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OXIDATIVE DEGRADATION OF SILK. II[1]

MINNIE LICHTE AND RACHEL EDGAR

From the Sections of Home Economics (Textiles and Clothing) and Chemistry, Iow Agricultural Experiment Station

Accepted for publication July 27, 1937

In order to compare the action on silks of permanganate in acidic solution with that in aqueous solution (2) similar plain-woven wild-silk fibroin and silk fibroin (table 1) and the same lead-weighted silk were analyzed for weight, ash, nitrogen, and wet strength, and the same iron-weighted, tin-weighted, tin-lead-weighted, and zinc-weighted silks for wet strength, after ten hours at 40°C. in fifty volumes of a range of concentrations of potassium permanganate, 0.06 *M* as to sulfuric acid. Before analysis the residual silks were freed of manganese dioxide in thirty minutes by fifty volumes of 0.05 *M* sodium hydrogen sulfite and washed in water until the rinse no longer reduced permanganate.

TABLE 1. *Analysis of fabrics*

	Wild-Silk pongee	Silk crepe
1. Weight, *ounces per square yard*	1.28	2.03
2. Thickness, *inch*	0.0042	0.0074
3. Yarns		
(a) Distribution		
Number per inch, warp	79	287
filling	67	103
Percentage by weight of fabrics, warp	49.1	58.9
filling	49.4	41.2
(b) Count, *thousand yards per pound, warp*	84.6	135.8
filling	70.7	81.4
(c) Twist, *number per inch, warp*	0	0
filling	0	54* (2) **
4. Breaking strength of fabric		
(a) Conditioned, *pounds per inch, warp*	24 (0.9) **	53 (2.3) **
filling	21 (0.5) **	32 (0.5) **
(b) Wet, *percentage of dry, warp*	79	72
filling	86	78
5. Elongation at breaking load, *percentage*		
(a) Conditioned, *warp*	27	34
filling	25	28
(b) Wet, *warp*	33	35
filling	31	35

* Two left-twisted yarns alternated with two right-twisted.
** Average deviation.

[1] Journal Paper No. J-467 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 262.

The fabrics were prepared for analysis as before except that the silk fibroins were extracted with petroleum ether instead of diethyl ether. The experimental procedures and methods of analysis were the same.

TABLE 2. *Weight, nitrogen, and wet strength of fabrics after ten hours in fifty volumes of 0.06 M sulfuric acid at 40°C.*

Fabric	Weight	Nitrogen	Breaking strength of wet warp
	percentage of original fabric		*pounds per inch*
A. Iron-Weighted silk crepe			26
B. Lead-Weighted silk crepe	91.2	8.64	19
C. Tin-Weighted silk crepe			19
D. Tin-Lead-Weighted silk crepe			19
E. Zinc-Weighted silk crepe			18
F. Silk crepe	99.9	18.47	33
G. Wild-Silk pongee	99.9	18.30	18

TABLE 3. *Weight of fabrics after ten hours in fifty volumes of oxidant at 40°C.*

Potassium permanganate	Sulfuric acid	Wild-Silk fibroin	Silk fibroin	Lead-Weighted silk
molarity	*molarity*	*percentage of acid-treated*		
0.0028	0.06	99.5	99.6	96.8
0.0054		99.3	99.3	96.4
0.0154		98.1	98.2	96.4
0.0214		97.3	96.8	
0.0337		96.1	96.1	
0.0404		94.8	92.3	

TABLE 4. *Ash of fabrics after ten hours in fifty volumes of oxidant at 40° C.*

Potassium permanganate	Sulfuric acid	Wild-Silk fibroin	Silk fibroin	Lead-Weighted silk
molarity	*molarity*	*percentage of acid-treated*		
0.0000	0.06	0.08	0.03	35.81
0.0028		0.16	0.06	36.71
0.0054		0.26	0.11	37.08
0.0154		0.49	0.19	37.36
0.0214		0.68	0.23	
0.0337		0.86	0.72	
0.0404		0.94	0.85	

TABLE 5. *Nitrogen of fabrics after ten hours in fifty volumes of oxidant at 40°C.*

Potassium permanganate	Sulfuric acid	Wild-Silk fibroin	Silk fibroin	Lead-Weighted silk
molarity	*molarity*	*percentage of acid-treated*		
0.0028	0.06	99.2	100.1	99.1
0.0054		99.1	98.7	98.3
0.0154		98.6	98.5	94.9
0.0214		97.5	98.0	
0.0337		95.5	96.3	
0.0404		94.0	93.6	

TABLE 6. *Wet strength of fabrics after ten hours in fifty volumes of oxidant at 40° C.*

Potassium permanganate	Sulfuric acid	A	B	C	D	E	F′	G′
molarity	*molarity*	*percentage of acid-treated*						
0.0028	0.06	85	84	79	74	83	91	94
0.0054		77	68	63	47	61	64	83
0.0154		77			26		45	67
0.0214							33	56
0.0337							3	44

DISCUSSION OF RESULTS

The similar percentile losses of weight and nitrogen by the wild-silk fibroin and silk fibroin in acidic permanganate (tables 2, 3 and 5) and wild-silk fibroin in aqueous permanganate suggest that superficial solution of these proteins has occurred rather than elimination of derivatives of different nitrogenous content. The greater loss in weight of silk fibroin than wild-silk fibroin in 0.0214 *M* permanganate may be explained by the lower ash (table 4).

Aqueous (3, 1) and acidic permanganate (4), displaced industrially by hydrogen peroxide as a bleach, are still commonly used in approximately tenth molar solution for the removal of stains from silk (6). Acidic solutions are shown more desirable for this because all the silks but the lead-weighted retained measurable wet strengths at concentrations double those at which their lowest strengths occurred in aqueous permanganate (table 6). This greater loss of strength by the silks and the greater loss of nitrogen by silk fibroin and lead-weighted silk in aqueous permanganate may be due in part to the resultant alkali (5, 7).

SUMMARY

1. Plain-woven fabrics of wild-silk fibroin, silk fibroin, and lead-weighted silk were analyzed for weight, ash, nitrogen, and wet strength, and plain-woven iron-weighted, tin-weighted, tin-lead-

weighted, and zinc-weighted silks for wet strength, after ten hours at 40°C. in fifty volumes of 0.0028 to 0.0404 *M* potassium permanganate, 0.06 *M* as to sulfuric acid.

2. The ash of the wild-silk fibroin, silk fibroin, and lead-weighted silk increased slightly with increasing concentration of oxidant.

3. Loss of strength exceeded that of protein. The strength of wild silk fibroin decreased less than that of silk fibroin; the iron-weighted lost less and the tin-lead-weighted more than the lead-weighted, tin-weighted and zinc-weighted silks which approximated that of silk fibroin at 0.0054 *M* potassium permanganate.

4. Percentile losses of weight by both wild-silk fibroin and silk fibroin, in aqueous and in acidic permanganate of the same molar concentration, agreed within experimental error.

5. Percentile losses of nitrogen by wild-silk fibroin and silk fibroin in acidic permanganate, and wild-silk fibroin in aqueous permanganate were the same as the percentile losses in weight, and were less than those of the lead-weighted silk. A greater loss of nitrogen by silk fibroin and lead-weighted silk and a far greater loss of strength by all the silks occurred in aqueous than in acidic permanganate.

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SEED IMPERMEABILITY AND VIABILITY OF NATIVE AND INTRODUCED SPECIES OF LEGUMINOSAE[1]

MARY ERNE-JEAN HUTTON AND R. H. PORTER[2]

From the Botany and Plant Pathology Section, Iowa Agricultural Experiment Station

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The production of impermeable seeds by plant species in a number of plant families, both cultivated and wild, has long challenged the attention of botanists throughout the world. The legume family is one of the most important in which seed impermeability is a characteristic condition.

Within recent years soil erosion has come to be recognized as a problem of national importance, the solution of which has an important bearing on the future well-being and prosperity of the people of the United States. Efforts to control erosion by mechanical methods have been only partially successful. Students of the erosion problem are rapidly recognizing that permanent plant cover on lands subject to erosion offers the most hopeful and effective method of prevention and control. Grasses have long proved their ability to hold soils and prevent both blowing and washing, but one of the most urgent problems today is to prevent not only further erosion but to rebuild lost fertility. This latter need must be met in part by the use of suitable legumes. A logical study, therefore, is to (1) determine what native species of the Leguminosae possess characteristics suitable for erosion control and (2) investigate methods of propagating such species. The propagation of any plant species is largely dependent on seed production and viability, and in the legume family seed impermeability is of decided importance.

In order to understand the methods of propagation exhibited by certain species of legumes native to or naturalized in Iowa, the investigations reported in this paper were undertaken.

PERTINENT LITERATURE

Inasmuch as an understanding of seed impermeability is dependent on a knowledge of the work of other investigators, a review of the general literature is presented here. Citations having a relationship to specific experiments will be discussed in the sections dealing with each experiment.

In 1916 Crocker (7) reported on the mechanics of dormancy in seeds. He found that dormancy is caused by the inhibition of one or more processes preceding or accompanying germination. He stated that seed coats are important because they do not always permit water to enter the seed and may prevent the exchange of oxygen and carbon dioxide. It is his opinion that after-ripening may effect changes in the seed coats as well as

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in the embryos, and that treatment with acids or bases, freezing and thawing, high temperatures and light may force germination.

Denny (10), who worked on the composition of membranes as related to permeability, found that the substances which determine permeability to water were lipoids, tanins, and pectic substances. He concluded that suberized layers were of little significance in the membranes studied and he could not detect the presence of soluble protein. Pammel (29), working with numbers of different legumes, found them all to possess a layer of cuticle which he thought prevented the entrance of water. In some cases the cuticle extended into the caps of the palisade cells. Raleigh (31) found that the Malphigian or palisade layer of *Gymnocladus dioica* contained pectic substances which he thought were the cause of impermeability.

Bergtheil (2) stated that impermeability of seeds of *Indigofera arrecta* was caused by the possession of a thin outer covering resistant to water. White (41), working with seeds of the same species, observed a structureless cuticle on the exterior of the seed coat. Rees (32) thought impermeability was caused by the presence of cutin either as an exterior membrane or in the walls of the palisade cells or both. Rogers (33) worked with seeds of *Amorpha nana* and found three layers in the seed coat, the two outer ones made up of cellulose interspersed with hemicellulose. He concluded that impermeability was probably brought about by some condition in the outer part of the outer layer of the seed coat.

Coe and Martin (6) decided that in sweet clover the light line was responsible and that its density was one of the chief causes of impermeability. Nelson (27) investigated the impermeability of seeds of sweet peas and other legumes and developed the idea that the pods of legumes deposited a varnish on the seed coats which caused them to become impermeable. Helgeson (16) was able to pick immature, permeable sweet clover seeds, hull them, and by subsequent drying, change the condition to one of impermeability, thereby suggesting that permeability and impermeability may be reversible. Hamly (14) concluded that impermeability in seeds of sweet clover is effected by the suberin caps of the Malphigian cells, but Denny states that suberization is not significant in creating an impermeable condition. Lute (20) found that in alfalfa impermeability was developed in the outer layer of palisade cells. In general, investigations have indicated that hard seed coats are caused by cutinization of the exterior of the seed coat or of the palisade layer as well.

Ewart (11) found that impermeability is important because it increases the life period of seeds. Impermeable seeds of many legumes were found to be viable after fifty years of storage, thereby providing a means by which plant species may survive over a period of time.

MATERIALS AND METHODS

Four general types of experiments have been carried on in this study. They may be classified under the following headings:

1. The viability of seeds after harvest and the effect of subsequent storage on viability and impermeability.
2. The correlation of the development of impermeability with stages of maturity.

3. Methods of reducing the percentage of impermeability in seed lots.

4. The determination of the region through which water first enters permeable seeds.

Seeds of *Strophostyles helvola* (L.) Britton., *Lespedeza capitata* Michx., *Lespedeza virginica* (L.) Britton., *Amorpha fruticosa* L., *Robinia pseudacacia* L. and *Glycine max* Merr. were used in the investigations herein reported. With the exception of *R. pseudacacia* and *G. max*, which are naturalized species, all of the plants studied are native to Iowa. Seeds of *S. helvola, L. capitata, L. virginica* and *R. pseudacacia* were harvested in 1933 while those of *A. fruticosa* were harvested in 1934. The only experiments, in which 1936 seed was used, are those dealing with the development of impermeability in immature seeds and studies with seeds of *G. max*. All of the seeds, excepting those of *R. pseudacacia* and *G. max*, were harvested in the pods and removed from them by hand to prevent injury to the seed coats. *R. pseudacacia* seeds were removed from the pods by machinery.

Germination tests were made in standard germinators, the seeds being placed at 20°C. for sixteen hours and at 30°C. for eight hours. Preliminary tests which were made at constant temperatures of 20°C. and 30°C., and at alternating temperatures showed that the germination temperatures used had little effect on the total germination of the seeds, but that at the alternating temperature the results were obtained in less time than at either of the constant temperatures. In all of the tests the seeds were placed between standard germination blotters such as are used in the seed laboratory and in one series of experiments additional plantings were made in sand and in the field in order to compare germination reactions in the field with those in the laboratory. Early tests were allowed to run for periods as long as three months, but it was found that there was a small percentage of germination after fifteen days and that the bulk of the germination occurred before the tenth day. Subsequent tests, therefore, were completed in from fifteen to twenty days. In making the germination readings four groups were recognized as follows: (1) normal seedlings, (2) abnormal seedlings, (3) dead seeds, and (4) impermeable seeds. Normal seedlings were those which to all appearances were capable of producing plants under reasonably favorable conditions. Abnormal seedlings included those with broken cotyledons, a shriveled radicle arrested in development, or a watery hypocotyl. Dead seeds were soft and mouldy. Impermeable seeds were hard, normal in color, and of normal size when the tests were completed.

The conditions described in the foregoing paragraph applied to all of the experiments which were carried on. Further discussion of procedures will be found in sections dealing with specific experiments.

EXPERIMENTAL RESULTS

THE VIABILITY OF SEEDS AFTER HARVEST AND THE EFFECT OF SUBSEQUENT STORAGE ON VIABILITY AND IMPERMEABILITY

The effect of storage on legume seeds is of considerable practical importance. In 1908 Ewart (11), who was working on seeds of a great number of plants, reported that some legume seeds germinated after a period

of fifty years and that those species with a large number of impermeable seeds germinated better at the end of that time than did those in which the seeds were all permeable.

Harrington (15) worked with a number of cultivated legumes, studying the impermeability at the time of harvesting and after storage. He found that freshly harvested red clover seed, hulled by hand, contained as much as 92 per cent impermeable seeds, and that the percentage of impermeability dropped only five per cent after one year of dry storage. Alsike clover contained 91 per cent hard seed when harvested and no seeds became permeable during the first year of storage. White clover contained 98 per cent hard seed when harvested and 90 per cent after one year. Sweet clover contained 98 per cent of impermeable seed at the time of harvesting and no reduction in impermeability occurred during the first year. Alfalfa, however, which contained only 32 per cent hard seeds when harvested had seven per cent hard seeds after one year. When seeds of these legumes were stored in manilla envelopes for five years, the impermeable seeds of red, white, alsike, and sweet clover became permeable very slowly although red clover seeds changed from impermeable to permeable somewhat more rapidly than the others investigated.

Goss (12) reported in 1924 on seeds of various legumes which had been buried in the soil in 1902. At the end of 21 years he found that 15 to 48 per cent of the seeds of *Lespedeza frutescens* and 27 per cent of the seeds of *R. pseudacacia* remained impermeable. Both red and white clover seed lots contained hard seeds at the end of the test. Helgeson (16) subjected seeds of white clover to three sets of storage conditions for three years: (1) out of doors, (2) room temperature in the laboratory, and (3) 7°C. at high humidity. He found that storage in the laboratory and in the cold room increased the number of impermeable seeds 12 per cent, while storage out of doors reduced the number of impermeable seeds 20 per cent.

Middleton (22) found that Korean Lespedeza possessed 20 to 50 per cent impermeable seeds when harvested, but that storage for five months reduced the amount of impermeable seeds to eleven per cent.

Stevens and Long (37) tested the germination of *Melilotus alba* and *M. officinalis* in 1912 and again in 1923.

In both cases the germination of the freshly harvested seed was extremely low in 1912 and the number of impermeable seeds was very high. The percentage of impermeable seeds remained quite high after eleven years.

Porter (30) found in tests of *R. pseudacacia* which were made within a few months after harvesting that the impermeable seeds numbered 77.5 per cent, that 22.5 per cent were soft, and that 18.25 per cent produced normal seedlings.

Lute (20) found that seeds of alfalfa became permeable in one year or less under storage conditions. Jones (17) was able to reduce the amount of impermeable seed in vetch by storing the seed in a humid atmosphere. Watt (39) found that storage under cool, dark conditions does not reduce the percentage of hard seed in sweet clover.

The following experiments were undertaken to determine the viability of seeds of the species used, after harvest and after a two-year storage period. Lots of 200 seeds each of *S. helvola, L. capitata,* and *L. virginica* were used. Germination tests were made in January of 1934 and

again in 1936. During the storage period the seeds were kept in cloth bags and were exposed to the changes in temperature and humidity of the laboratory. Germination was classified as N, normal; A, abnormal; D, dead; and H, hard. The results are given in table 1.

TABLE 1. *A comparison of the germination of S. helvola, L. capitata and L. virginica in 1934 and 1936*

Germ.	*S. helvola*		*L. capitata*		*L. virginica*	
	1934	1936	1934	1936	1934	1936
N	33.0	37.0	3.0	4.5	8.5	3.5
A	0.0	0.0	0.0	0.0	0.0	0.0
D	12.0	33.0	8.5	26.0	11.5	21.5
H	55.0	30.0	88.5	69.5	80.0	75.0

In general, the data presented in table 1 show that the percentage of impermeable seed is high in the species tested at the time of harvest, that during storage impermeability is gradually reduced, and that unless suitable growing conditions are provided when the seeds become permeable they soon lose their vitality.

CORRELATION OF THE DEVELOPMENT OF IMPERMEABILITY IN SEEDS WITH STAGES OF MATURITY

Little work has been done on the relation of moisture content of developing seeds to their impermeability although for many years it has been known that impermeability develops after seeds have reached a certain stage of maturity. Watt (39) found in *Meliotus alba* that the light line appeared within sixteen days after pollenization and that the seed coat was mature in twenty days. Raleigh (31) mentions in the case of *Gymnocladus dioica* that impermeability of the seed coat seems to be caused by a general shrinking and condensation of the seed coat in drying.

Helgeson (16) tested the germination of lots of immature seed of sweet clover which he distinguished by the terms "yellow pod" and "brown pod". Both types of seed had higher moisture percentages than ripe seed, that of the "yellow pod" lots being higher than that of the "brown pod" lots. His investigations showed that seeds from the "yellow pod" lots germinated 100 per cent in ten days, while seeds from the "brown pod" lots contained 43 per cent impermeable seed at the end of that time. Mature seed showed 86.5 per cent impermeable seeds.

Harrington (15) performed some experiments dealing with the influence of maturity on the rate of softening of impermeable seeds of red and alsike clover. He found that in lots of rather immature red clover 78 per cent of the impermeable seeds softened in one month and 100 per cent softened in 13 months. In the lots of well matured red clover seed only five per cent of the impermeable seeds softened in one month and 44 per cent softened after three years. Alsike clover showed a similar condition.

The experiments described below were planned and executed for the purpose of determining what relationship, if any, exists between the impermeability and relative maturity of seeds in samples of *S. helvola*. The

index of maturity used was the percentage of moisture in the seeds immediately after harvest.

Seed pods of *S. helvola* were gathered from plants in the field in September, 1936, placed in manilla envelopes and carried to the laboratory in a vasculum. Each envelope was removed in order, and the seeds shelled by hand, after which they were divided into three groups. Seeds in the first group were bright green in color, and although they seemed firm and well developed they were considerably larger than mature seeds. Seeds of the second group showed darkening around the hilum and were developing the pubescence or scaliness which characterizes the seed coats of mature seeds. Seeds in the third group were dark brown in color, very pubescent, and produced a ringing sound when dropped on glass. They were about the size of normal, mature seeds. Each lot of seed was divided into two portions, one of which was tested for germination, the other for the percentage moisture. Samples used for moisture tests were weighed, dried for 48 hours at 100°C., then reweighed. All of the moisture percentages were calculated on the green weight basis. The results of these experiments are given in table 2.

TABLE 2. *Comparison of moisture percentage, appearance, and germination of seeds of S. helvola at different stages of maturity*

Sample	Appearance	Green wt. grams	Dry wt. grams	Pctg. moisture	Number seeds	Pctg. germination			
						N	A	D	H
1	Green Firm	7.731	2.096	57.87	190	64	1	35	0
2	Hilum Dark	8.946	5.096	43.04	200	100	0	0	0
3	Dark Hard	8.844	7.059	20.18	200	48.5	0	0	51.5

In general, it may be concluded from the data in table 2 that when seeds of *S. helvola* contain 57.87 per cent moisture the embryos of many seeds are too immature to germinate, that at 45 per cent moisture they are mature and germinate readily, and that when the moisture percentage falls below 21 per cent impermeability develops rapidly.

METHODS OF REDUCING THE PERCENTAGE OF IMPERMEABILITY IN SEED LOTS

For many years botanists, agronomists and seed analysts have been interested in methods of reducing the number of impermeable seeds in seed lots. Many methods have been tried with varying degrees of success. The most important of these have been scarification and treatment with sulfuric acid. Scarification of seeds of cultivated legumes by means of abrasives has long been practiced by seedsmen, but it has been found that certain kinds of machines increase greatly the number of abnormal sprouts and dead seeds. Graber (13) states that although scarification increases immediate germination it decreases longevity of alfalfa seeds. Nelson (24), working with red clover, found that abrasion methods of reducing the hard seed content of seed lots caused broken cotyledons and

that damage was greatest in large seeded lots. He indicated (26) that the injury might not be so important in the field as it was in the laboratory. He stored scarified seed (25) in dry and moist conditions and found that at the end of 400 days those stored dry had from 33 to 43 per cent dead seeds, whereas, those kept in a moist condition contained 23 per cent dead seeds. The more severe the abrasion the greater the loss in vitality of the seeds stored dry. This indicates that breaking of the seed coats by abrasion may permit the seeds to lose moisture and so kill them in addition to the danger of destroying the embryos. Stewart (26) stated that scarification increased germination but that most of the increase was represented by abnormal seedlings. Rose (34) suggested scarifying seeds by blowing them against a bank of needle points. This method had the advantage of not breaking the embryos and did increase the percentage germination of hard seeded lots.

Treatment of seeds with sulphuric acid is a method of reducing impermeability which also has been in use for some time. Love and Leighty (19) were able to raise the germination of red, white, sweet, alsike and Japan clover by treatments with concentrated sulphuric acid for not more than one hour. Any longer period caused injury. Jones (17) was able to destroy the impermeability of vetch seed by use of sulphuric acid and determined that it destroyed the cuticle and the outer layer of the Malphigian cells. Rogers (33), working with *Amorpha nana,* hastened germination by several days and slightly increased total germination by subjecting seeds to 80 to 100 per cent concentrations of sulphuric acid for five to seven minutes. Thornber (38) found that a mixture of sulphuric acid and chromic acid is effective in reducing impermeability in seeds of Robinia, Gleditsia and Gymnocladus.

Many experiments have been performed to determine the effect of temperature on hard seeds. Both high and low temperatures have been investigated. Thornber (38) stated in 1904 that seeds of Acacia germinated readily when submitted to treatment with water at 85°C. to 88°C. for two to six minutes. McNair (21) increased the germination of *Trifolium reflexum* seed 60 per cent by boiling it for 60 seconds. By first soaking it in cold water for twelve hours and then boiling it for 60 seconds he was able to increase the germination to 93 per cent. Breasola (3), while working on a method to destroy dodder seeds in lots of legume seed, found that heating to 75°C. for one hour did not kill the dodder seeds and in some cases improved their germination by reducing the percentage of hard seed. An anonymous note (1) gives a treatment with hot water at 50°C. to 70°C. as a method of bringing about the germination of hard seeds. In 1925 Staker (35) investigated the effect of dry heat on the germination of alfalfa. He found that heating at temperatures between 60°C. and 90°C. increased the germination by reducing the percentage of impermeable seeds. Seeds which were low in vitality were killed by this treatment. His results are in accord with those of Stewart (36), who treated seeds of alfalfa at a temperature of 85°C. for four hours and increased germination, and those of Lute (20), who increased germination of alfalfa 25 per cent by a treatment of 80°C. for two hours. Lute found that if seeds were treated for over five hours the percentage of germinating seeds was lessened. Rogers (33) was unable to cause a reduction of hard seeds in *A. nana* by the same methods. Hamly (14) found that permeability of the seed coat

could be brought about in sweet clover by a moderate heating. Harrington (15) found that with dry heat at 45°C. he got a small reduction in the percentage of impermeable seeds.

Some work has been done on the effect of freezing and sub-freezing temperatures on the permeability of seeds. In 1898 Brown and Escomb (4) reported that temperatures as low as —198°C. and —192°C. had no effect on the germination of impermeable seeds. In 1916 Harrington (15) mentioned that impermeable seeds of legumes resist freezing, and stated that if seeds do respond to freezing in a wet condition the permeable seeds are killed. In 1928 Midgley (23) worked on the effect of alternate freezing and thawing on the impermeability of alfalfa seed and found that the first freezing reduced the number of impermeable seeds 23 per cent and that subsequent thawing and freezing had little or no effect. He found that freezing dry seed was as effective as freezing seeds in a moist environment. Busse (5) in 1930 reported on the effect of freezing seeds of alfalfa and sweet clover in liquid air. A temperature of —190°C. caused the seeds to become permeable and did not cause any injury to the seeds. A temperature of —80°C. brought about a permeable condition in alfalfa but had no effect on sweet clover. When Rogers tried freezing experiments on seeds of *A. nana* no reduction of impermeability was obtained.

Davies (8) has studied the effect of high pressures on impermeability. He increased the germination of alfalfa and sweet clover 30 per cent by exposing the seeds to 500 to 2,000 atmospheres of pressure at 18°C. for five and ten minute periods. He found that exposure to high pressures for short periods of time was more desirable than exposure to low pressures for longer periods.

The latest method of reducing impermeability in legume seeds is that of shaking, which was first used by Hamly (14) in 1932. He placed seeds of *M. alba* in a 500 cc. Florence flask and shook them for ten minutes at three oscillations per second, thereby raising the percentage of permeable seeds from 0.5 per cent to 91.0 per cent. Porter (30) used this method on seeds of *R. pseudacacia* and found that shaking for twenty minutes in a two-liter glass bottle increased the percentage of germinating seeds from fifteen to ninety per cent.

Of the types of methods for reducing the impermeability of seeds discussed above the following were studied in the investigations recorded here; namely, (1) heat treatments at 65°C. and 85°C. for varying periods, (2) freezing and thawing with various modifications, (3) scarification by means of abrasives and sulphuric acid, and (4) shaking for different periods of time.

Seeds of *L. capitata, S. helvola,* and *A. fruticosa* were treated by the methods mentioned in the foregoing paragraph. Reactions of shaken and unshaken seeds of these species, and of *L. virginica* and *R. pseudacacia* in blotters, sand and field conditions were studied as well.

Each group of seeds which was subjected to temperatures of 60°C. and 85°C. for varying periods was divided into two lots, one of which remained dry throughout the treatment while the other was covered with water. The seeds were placed in small bottles. Those which were subjected to moist heat were covered with tap water and were thorougly shaken to prevent any from floating. All of the bottles were unstoppered throughout the treatment. After preparing the seeds, the bottles were

placed in constant temperature ovens running at 65°C. and 85°C. Bottles were removed from the 85°C. oven at intervals of 1, 2, 3, 5 and 7 hours, while those in the 65°C. oven were left for 6 or 8 hours. The seeds were allowed to cool after treatment, excess moisture was removed from the wet lots, and lots of 100 seeds each were placed in the germinators at once. As many replications were made as the number of seeds in the sample permitted.

The seeds which were subjected to freezing and thawing were placed in small bottles and covered with water. One set was aspirated to remove any small air bubbles which might cling to the seed coats and especially to the hilums, thus preventing water from penetrating the interstices. A second set was washed 15 minutes in 95 per cent ethyl alcohol for the same reason. Both sets were then covered with fresh tap water and frozen at a constant temperature of —6.5°C. After sixteen hours of this treatment they were removed to a warmer temperature until all of the ice had melted. They were then subjected to the freezing temperature a second time for about eight hours. Immediately after the treatment was completed the seeds were dried between blotters, counted and placed in the germinator.

Scarification by means of sandpaper and sulphuric acid was accomplished as follows: Seed that were scarified by sandpaper were placed in a small box which was lined with grade one-half sandpaper. A wooden block covered with the same material was moved back and forth on top of the seeds for approximately two minutes. Only slight pressure was applied so that the embryos would not be too severely injured. After the treatment, the seeds were counted and placed in a germinator. A number of normal seeds were placed in concentrated sulphuric acid for periods of 10 and 30 minutes. They were then washed through clear water several times to remove any traces of the acid and were placed in the germinator at once.

Shaking treatments which were applied to seeds of the various species were based on the treatment developed by Hamly as applied by Porter. Small quantities of seed were placed in a quart fruit jar and covered tightly. The container, with the linear axis parallel to the earth's surface,

TABLE 3. *The effect of different treatments on germination of seeds of L. capitata*

Treatment	No. seeds	Percentage germination			
		N	A	D	H
Check	400	4.25	0.0	17.75	78.00
Shaken 10 minutes	200	83.50	0.0	14.50	1.00
Shaken 20 minutes	200	84.00	0.0	16.00	0.00
Frozen and thawed	400	2.00	0.0	13.25	84.75
Washed in alcohol, frozen and thawed	400	3.00	0.0	12.50	84.50
85°C. 1 hour wet	400	7.25	0.0	13.00	79.75
85°C. 1 hour dry	300	5.00	0.0	11.33	83.67
85°C. 2 hours wet	200	5.50	0.0	11.00	83.50
85°C. 2 hours dry	200	5.50	0.0	11.50	83.00
85°C. 3 hours wet	300	7.33	0.0	10.00	82.67
85°C. 3 hours dry	200	5.50	0.0	21.50	73.00

was shaken up and down by hand for periods of 10 and 20 minutes at a uniform rate of speed. Germination tests were started as soon as the treatment was completed. For each species studied check lots of untreated seeds were tested in the germinator at the same time as the treated lots.

The data in table 3 show that of the treatments tried on seeds of *L. capitata* the only ones which were effective in reducing the percentage of impermeable seed were those involving shaking.

The data in table 4 show that for *S. helvola* the only treatment of those tried which increased the number of normal seedlings was sulphuric acid. The shaking method of treating impermeable seeds as applied to *S. helvola* produced no significant changes in the response of the seeds as measured by a germination test with the possible exception of the treatment for ten minutes.

By reference to the data in tables 3 and 5 it may be noted that shaking was the most effective means of increasing the percentage of normal seedlings in *A. fruticosa* as it was with *L. capitata*.

In comparing the reactions to treatments of the three species tested several conclusions may be drawn. The most effective method of reducing impermeability in *L. capitata* and *A. fruticosa* is that of shaking. Shaking had no effect on seeds of *S. helvola*.

Freezing and thawing as applied in these experiments had no effect on the seeds of any of the species tested. Scarification by sandpaper and treatment with sulphuric acid were effective in reducing the percentage of impermeable seeds in the species which were treated in this way, but abrasion methods greatly increased the number of abnormal sprouts.

TABLE 4. *The effect of different treatments on the germination of seeds of Strophostyles helvola*

Treatment	No. seeds	Percentage germination			
		N	A	D	H
Check	300	29.0	0.7	12.7	57.6
Shaken 10 minutes	300	37.7	3.0	8.0	51.3
Shaken 20 minutes	300	31.3	1.3	10.7	56.7
Frozen and thawed	196	11.2	0.5	30.1	58.2
Washed in alcohol, frozen and thawed	177	3.95	0.0	35.0	61.05
85°C. 1 hour wet	150	26.7	1.3	14.0	58.0
85°C. 1 hour dry	200	31.5	1.5	11.0	56.0
85°C. 2 hours wet	150	15.4	0.0	18.0	66.6
85°C. 2 hours dry	150	24.6	0.6	11.4	63.4
85°C. 5 hours wet	100	3.0	0.0	58.0	39.0
85°C. 5 hours dry	100	26.0	1.0	11.0	62.0
85°C. 7 hours wet	100	2.0	0.0	61.0	37.0
85°C. 7 hours dry	100	23.0	0.0	32.0	45.0
65°C. 6 hours wet	150	2.7	0.0	39.3	58.0
65°C. 6 hours dry	200	28.0	0.5	17.0	54.5
65°C. 8 hours wet	150	6.0	0.0	45.0	49.0
65°C. 8 hours dry	200	27.0	0.5	6.5	66.0
H_2SO_4 ten minutes	100	44.0	0.0	15.0	41.0
H_2SO_4 thirty minutes	200	79.0	1.0	15.0	5.0

TABLE 5. *The effect of different treatments on the germination of Amorpha fruticosa*

Treatment	No. seeds	Percentage germination			
		N	A	D	H
Check	350	46.00	0.00	30.00	24.00
Shaken 10 minutes	400	73.00	0.00	26.75	0.25
Shaken 20 minutes	300	71.00	1.30	26.70	0.00
Frozen and thawed	400	55.50	1.00	26.00	17.50
Washed in alcohol, frozen and thawed	400	49.75	1.00	32.75	16.50
Sandpapered	200	48.50	11.50	40.00	0.00
85°C. 1 hour wet	175	47.43	0.57	37.14	14.86
85°C. 1 hour dry	200	29.00	0.00	31.00	40.00
85°C. 2 hours wet	285	35.00	1.05	50.00	13.90
85°C. 2 hours dry	300	31.66	1.00	26.00	41.34
85°C. 3 hours wet	300	11.33	0.67	80.66	7.34
85°C. 3 hours dry	300	33.00	1.66	19.34	46.00
85°C. 5 hours wet	92	0.00	0.00	98.91	1.00
85°C. 5 hours dry	100	11.00	1.00	51.00	27.00
85°C. 7 hours wet	200	0.00	0.00	100.00	0.00
85°C. 7 hours dry	100	14.20	2.00	53.00	31.00
65°C. 6 hours wet	400	0.00	0.50	98.50	1.00
65°C. 6 hours dry	200	28.50	1.50	41.00	29.00
65°C. 8 hours wet	400	0.50	0.00	96.00	3.50
65°C. 8 hours dry	188	22.40	0.50	43.00	34.10

The effect of dry heat on some of the species tested, particularly *A. fruticosa,* suggests that impermeability may be a reversible process in part. That is, if permeable seeds are dried to a certain point, shrinkage of the seed coats may occur, causing an impermeable condition to develop. However, if impermeability is destroyed by a mechanical means such as shaking it is not reversible under storage conditions in the laboratory. Shaken seeds of *L. capitata* germinated as well six months after storage as they did when first shaken. This view reconciles that of Hamly (14), who states that impermeability is an irreversible condition, with those of other investigators who suggested that impermeability is reversible. Heat applied to wet seeds was detrimental in all cases excepting that of *L. capitata.*

IMPERMEABILITY IN SOYBEAN SEED

In January, 1937, a sample of Wilson soybeans received at the seed laboratory showed 40 per cent impermeable seeds. Following the germination test, 146 seeds, which had remained impermeable in moist towels, were divided into two lots, one with 100, the other with 46 seeds. The first lot was placed in a glass bottle and shaken for 10 minutes, then planted in moist, sterile sand. The second lot of 46 seeds was planted in sand without further treatment. At the end of 10 days 96 per cent of the shaken seeds had produced normal seedlings and after 25 days 10.9 per cent of the check lot produced normal seedlings. Both lots were held at 30°C. constant temperature. Untreated seed of this lot planted in the field in early June, 10 replications, gave 76.3 per cent stand.

In May, 1937, a sample of Cayuga soybeans was obtained from the Agronomy Department, Iowa State College, which showed nearly 80 per

cent impermeable seeds. In June two sub-samples, each containing 600 seeds, were prepared from each of the bulk lots of Wilson and Cayuga beans referred to above. One sub-sample of each variety was kept for a check, the other shaken 10 minutes in a glass fruit jar. Each sub-sample was then divided into two equal portions of 300 seeds and one was planted in sterile moist sand held at a temperature of 33°C., the other in paper towels kept at alternating temperatures of 20°-30°C. The results of the several tests after six days are shown in table 6.

TABLE 6. *Effect of impaction on impermeable seeds of soybeans*

Variety	Germination									
	Check					Shaken				
	Towels				Sand	Towels				Sand
	N	A	D	H	N	N	A	D	H	N
Cayuga	21	2	7	70	24	42	3	8	47	70
Wilson	75	0	2	23	76	87	0	2	11	90

From the data in table 6 it is evident (1) that the percentage of impermeable seeds in Wilson soybeans was reduced from 40 in January to 23 in June, 1937, while stored in the laboratory at room temperature, and (2) impaction by shaking in a hard walled container is an effective method of reducing the hard seed content of soybean seeds. The most marked reduction was in the Cayuga variety, in which the percentage of normal seedlings in sand was increased from 24 for the check to 70 for the treated lot. The reason for the significant difference in normal seedlings obtained in towels and sand for the treated lot of Cayuga seeds is not known. Similar differences in untreated lots of okra, black locust and other soybean seeds have been noted in laboratory tests which indicate that sand may be a superior substratum for testing species with impermeable seeds.

COMPARISON OF FIELD AND LABORATORY GERMINATION

When it was found that shaking had an effect on the impermeability of seeds of some of the species of legumes studied, it was considered advisable to determine the respective germination performance of seeds of several species in blotters, in sand, and in the field. Seeds of *S. helvola, L. capitata, L. virginica, A. fruticosa* and *R. pseudacacia* were included in the tests. Four lots of seeds of *R. pseudacacia* from different parts of the United States were used. Only one seed lot of each of the other species was available. Seeds were shaken in the manner previously described for twenty minutes and were then prepared for planting. Laboratory tests were based on the germination of 200 seeds in each set of conditions in all species excepting *R. pseudacacia* and field tests were based upon the response of 400 seeds for each treatment. In the case of *R. pseudacacia* different numbers of seeds were used, running as high as 1,107 for one treatment. Blotter tests followed the same procedure as was used in previous experiments. Seeds which were tested for response in sand in the germinator were placed in square paper boxes containing one-half inch of fine quartz sand which had been previously sterilized by heating for

four hours. They were then covered with sand, seeds of the small seeded species being covered with a quarter inch layer and those of *S. helvola* and *R. pseudacacia* were covered by a thick layer. The field plantings were made about the middle of April. Seeds of the first four species named above were planted in five-foot rows, 100 seeds per row with four rows in consecutive order. Seeds of *R. pseudacacia*, 100 per row, each repeated six times, were planted according to a restricted random arrangement such that each sample appeared once in each block and once in each column. Table 7 shows the response of seeds at each set of conditions.

The data in table 7 show that the shaking treatment reduced impermeability in all of the species tested excepting *S. helvola* and that an increase in germination occurred in sand, in blotters and under field conditions.

TABLE 7. *A comparison of the germination of shaken and unshaken seeds of S. helvola, L. capitata, L. virginica, A. fruticosa and R. pseudacacia in blotters, sand and field conditions*

Species and treatment	Blotter				Sand	Field
	N	A	D	H	N	N
S. helvola						
shaken	37.7	3.0	8.0	51.3	32.5	22.0
check	37.0	0.0	33.0	30.0	27.0	12.0
L. capitata						
shaken	85.5	0.5	14.5	1.5	80.5	48.0
check	4.5	0.0	26.0	69.5	4.5	2.0
L. virginica						
shaken	33.0	0.5	32.5	34.0	31.0	10.0
check	3.5	0.0	21.5	75.0	4.0	1.0
A. fruticosa						
shaken	67.0	0.0	32.5	0.5	53.0	12.5
check	45.0	0.0	30.0	25.0	50.0	1.0
R. pseudacacia						
Lot A. shaken	77.0	22.5	0.0	0.0	79.0	51.0
check	38.0	0.0	0.0	62.0	34.5	21.0
Lot B. shaken	61.0	12.0	18.0	9.0	74.0	45.2
check	28.5	0.0	7.5	64.0	19.5	11.4
Lot C. shaken	82.2	1.9	3.1	12.8	86.5	52.0
check	12.8	0.2	2.1	84.8	6.5	6.5
Lot D. shaken	57.0	20.4	5.6	17.0	54.0	33.2
check	45.5	0.0	6.5	48.0	41.0	25.4

DETERMINATION OF THE REGION THROUGH WHICH WATER FIRST ENTERS SEEDS WHEN THEY BECOME PERMEABLE

The question of whether or not legume seeds possess a special structure which facilitates the entrance of water, or whether part or all of the impermeable seed coat becomes permeable, is a problem which has long interested investigators. White (41), Rees (32), Jones (17) and Rogers (33) agreed that the impermeability of the seeds with which they worked was brought about either by the structure of the Malphigian layer or by

the cuticle formed on the surface of the seeds. Coe and Martin (6) found that there was no chemical difference between the seed coats of permeable and impermeable seeds of sweet clover but that the walls of the Malphigian cells were more highly developed in impermeable seeds. None of these authors discovered any means by which water might enter the seeds other than through the seed coat.

In 1932 Hamly (14), working with sweet clover, first used a method of treating impermeable seeds based on the principle of an impact or blow to each seed repeated a large number of times. This method has been described earlier in this paper. Having found that shaking reduced the percentage of impermeable seeds, Hamly soaked seeds softened in this way in one per cent solution of osmic acid and found that darkening occurred first at a point on the side of the hilum opposite the microphyle. This point is known as the strophiole. From the strophiole the darkening spread through the seed, following a path which would be taken by water upon its entrance into the seed. When he sectioned seeds of sweet clover treated in this manner, he found a cleft at the strophiole around which the tissues were darkened, the darkened region extending into the embryo. From these data he concluded that the impact of shaking sweet clover seeds separates cells of the strophiolar region, thus permitting the entrance of water.

In the following experiments, seeds of *A. fruticosa*, *L. capitata* and *S. helvola* were studied. After being subjected to different treatments to reduce impermeability, seeds of the three species were placed in an aqueous solution of Mayer's hemalum. They were left at a constant temperature of 30°C. for twelve hours, after which interval the first signs of swelling appeared. They were then removed to water and washed thoroughly, after which freehand sections were made of some seeds. Other seeds were killed in 80 per cent alcohol and the ends opposite the hilum clipped to facilitate the entrance of reagents into the seeds. None of the reagents used contained any acid, since acid would have destained the tissues. After killing, the seeds were carefully dehydrated in butyl alcohol and were then infiltrated with paraffin. In order to soften the tissue and facilitate cutting, blocks of paraffin containing the seeds were soaked in water at 30°C. for twelve hours. Serial transverse sections were cut fifteen microns thick. Studies were made of the external appearance of the seeds in question, in order to ascertain what position was occupied by the hilum with relation to the curvature of the seed. Seeds of *A. fruticosa*, *L. capitata* and *S. helvola* are illustrated in figures 1, 2 and 3, respectively.

Seeds of *A. fruticosa* which had been subjected to wet heat, dry heat, freezing and thawing, shaking and sandpapering, were sectioned and studied, and shaken seeds of *L. capitata* also were included. Preliminary freehand cross sections of shaken seeds of *A. fruticosa* stained in an aqueous solution of gentian violet showed that the seeds were covered with a thin coating of material, presumably cutin, which stained very heavily with gentian violet. The base of the hilum was the only point at which the stain passed through the layer of thick-walled cells immediately beneath this coating. A cone-shaped area extending into the inner portions of the seed coat from the base of the hilum depression was heavily stained.

When thin sections of paraffin material were studied it was found that in addition to the thin cuticular coating, which also stained with hemalum, the seed coat was composed of three distinct layers. The cells immediately beneath the cuticle composed the Malphigian layer and possessed very thick walls with small lumina which were widest at the base, tapering toward the outer wall. Beneath the Malphigian layer a layer of osteosclereid cells was found. The innermost layer of the seed coat was composed of nutrient cells. No stain passed through the Malphigian layer of seeds which were shaken.

The hilum of seeds of *A. fruticosa* is located in a rather deep depression near the narrow end on the incurved surface. The depression is filled with disorganized abscission tissue which stained heavily with hemalum. At the base of the hilum an opening occurs in the Malphigian layer. This opening is occupied by the vascular bundle which connects the seed to the parent plant. It is at this point in seeds of *A. fruticosa* that a break occurs when the seeds are shaken. When seeds of *A. fruticosa* were shaken a cleft appeared through the vascular bundle, the adjoining tissue of which stained slightly with hemalum, and a faint color was found at the end of the bundle next to the embryo. No other break occurred in the seed coat. It was not possible to make sections of hard seeds for comparison, but when seeds were scarified with sandpaper, stained and sectioned, it was found that the seed coat was broken in a number of places. In some of the seeds studied the broken seed coats were accompanied by a cleft at the hilum, but this did not occur in all cases.

Seeds that were made permeable by wet heat showed characteristics in cross section which were similar to those induced by freezing. In both cases the whole seed became permeable although in many cases no definite breaks occurred. All of the tissue in the hilum region was stained. Seeds which became permeable as a result of treatment with dry heat showed much the same type of reaction as did shaken seeds.

Cross sections of seeds of *L. capitata* showed the same type of seed coat and the same arrangement of tissues at the hilum as was found in seeds of *A. fruticosa*, although the hilum depression was not so deep. In this case, too, a cleft occurred through the vascular bundle which permitted the inner tissues of the seed coat to become stained.

Seeds of *S. helvola* gave unsatisfactory results when sectioned because the size of the seeds resulted in poor infiltration with paraffin. No reliable observations as to staining were made.

SUMMARY

In the preceding pages are recorded the results of investigations dealing with the viability, longevity, impermeability and germination of seeds obtained from six species of Leguminosae. The plant species whose seeds were investigated are *Lespedeza capitata, L. virginica, Amorpha fruticosa, Strophostyles helvola, Robinia pseudacacia,* and *Glycine max.* The following conclusions are based on the experimental data obtained.

1. At the time of harvest the percentage of impermeable seeds was found to be 55, 88 and 80 for *S. helvola, L. capitata* and *L. virginica*, respectively. Storage for two years in the seed laboratory at Ames, Iowa, had little or no effect on the seeds of *L. virginica*, but seeds of *L. capitata*

and *S. helvola* showed a significant reduction in percentages of impermeable seeds and a corresponding increase in non-viable seeds.

2. Development of impermeability in young seeds of *S. helvola* is directly correlated with the moisture content of the seeds, increasing rapidly after the moisture percentage drops below 21.

3. The most effective method of reducing the percentage of impermeability in seeds of *L. capitata* and *A. fruticosa* is that of shaking in a glass bottle for ten minutes. In samples treated by this method no impermeable seeds remained. Seeds of *S. helvola* were unaffected by the treatment.

4. Shaking for twenty minutes greatly increased the production of normal seedlings of *L. capitata, L. virginica, A. fruticosa* and *R. pseudacacia* under both laboratory and field conditions and is the most satisfactory method so far known for increasing germination in these species when impermeable seeds are present in high percentages.

5. Shaking for ten minutes in a glass bottle is effective in reducing the percentage of impermeable seeds in two varieties of *Glycine max*, namely Cayuga and Wilson.

6. Impermeability of seeds of *S. helvola* is reduced most effectively by treatment with concentrated sulphuric acid for thirty minutes. No abnormal seedlings were produced.

7. Heating in a wet or dry condition, scarifying with sandpaper, and freezing and thawing did not increase the percentage of normal seedlings produced by *L. capitata, S. helvola* and *A. fruticosa*. Temperatures of 65°C. and 85°C. for periods of three hours or more materially increased the percentage of dead seeds of *A. fruticosa* when the seeds were treated in water. None of the temperature treatments affected impermeable seeds of *S. helvola*.

8. Shaking seeds of *A. fruticosa* and of *L. capitata* causes fissures to appear at the base of the hilum depression, through which water enters the seeds, based on the entrance of a dye as a criterion for the entrance of water prior to germination. Heating dry seeds of *A. fruticosa* at 85°C. for five to seven hours has the same effect as shaking.

9. The observations suggest that repeated blows during the shaking process may cause fissures to develop at the base of the hilum, which point is probably less able to withstand the stresses produced. The form of the hilum in seeds of *A. fruticosa* and of *L. capitata* is somewhat U-shaped and a blow at both extremities simultaneously might cause separation of the cells at the base.

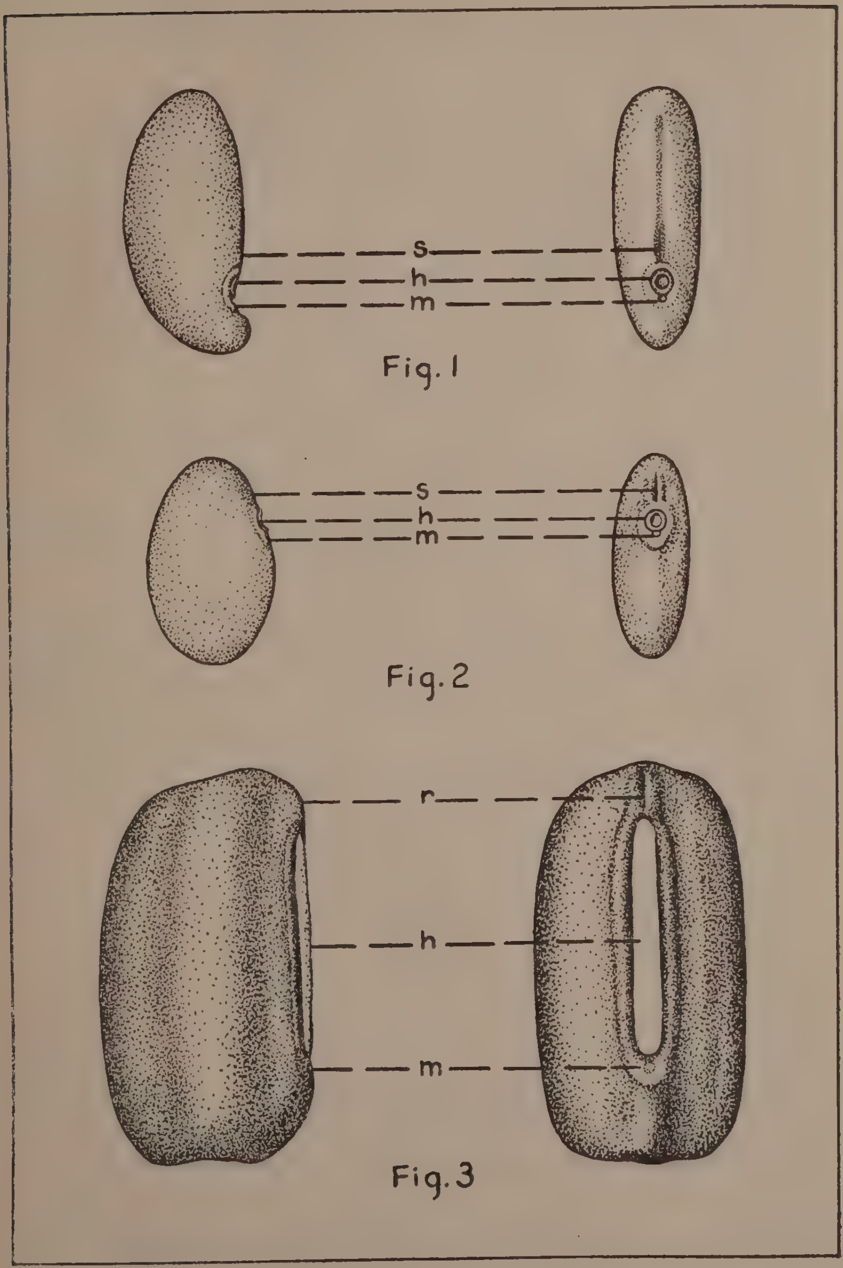
PLATE I

Fig. 1. Diagram of a seed of *A. fruticosa*.
x 12. left—side view; right—hilum view; s—strophiole; h—hilum; m—micropyle.

Fig. 2. Diagram of a seed of *L. capitata*.
x 12. left—side view; right—hilum view; s—strophile; h—hilum; m—micropyle.

Fig. 3. Diagram of a seed of *S. helvola*.
x 12. left—side view; right—hilum view; r—raphe; h—hilum; m—micropyle.

PLATE I



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STROPHOSTYLES HELVOLA (L.) BRITTON, ITS HABITS AND PROBABLE VALUE ON ERODED AREAS[1]

JOHN N. MARTIN

From the Botany and Plant Pathology Section, Iowa Agricultural Experiment Station

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GENERAL CHARACTERISTICS

Strophostyles helvola, commonly called Trailing Wild Bean, is an annual legume common throughout Iowa and other midwestern states, its range reaching westward into the Dakotas and southwest into Texas and eastward to the Atlantic, but is generally confined in its distribution to sandy soils and its habitat is given in manuals as sandy shores and river banks. The collections in the Iowa State College herbarium show that it occurs in the various sections of Iowa. According to Pammel (7) it is generally abundant in the river valleys and sandy areas of the state. With the *Strophostyles helvola* there is commonly associated another annual species, *S. pauciflora* (Benth.) Wats, which is less common in Iowa, inferior in size, but similar in characteristics and habits.

The plants of the genus *Strophostyles* are similar to the true beans (Phaseolus), differing from the beans mainly in not having a twisted keel. By Persoon the plants of this genus were classed as beans, *Strophostyles helvola* being called *Phaseolus diversifolius.* Their close relationship to the beans suggests the possibility of combining the desirable features of the genera in crosses.

The vines of *Strophostyles helvola,* ranging up to two meters in length, twine around corn, alfalfa, and other field crops, and often form mats so dense over the surface of the ground that they interfere with cultivation (fig. 1). It is claimed by some farmers that this plant makes good hay and no doubt it adds much nitrogen to the soil, for it bears an abundance of exceptionally large nodules. The virtues of this plant probably more than compensate for its somewhat weedy habit. In a bulletin on the wild legumes of Maryland and their utilization, Norton (5) states that the *Strophostyles helvola* approaches the cowpea in feeding value and has great promise.

The organism associated with its nodules is not known and according to cross inoculation studies at the Illinois Experiment Station (2) it does not cross inoculate with any of the common cultivated legumes. The formation of nodules begins early in the life of the plant, while the radicle is but a few inches in length. and before the plant is a foot in height the nodules are abundant and exceptionally large (fig. 1). No evidence of the lack of the organism has been observed in any of the various habitats where the plant has been found growing, but the tolerance of the organism of soil conditions, especially of acidity, may be a limiting factor of some importance in the distribution of this legume.

Strophostyles helvola is exceedingly prolific, flowering continuously from June or early July until frost and producing an abundance of seeds.

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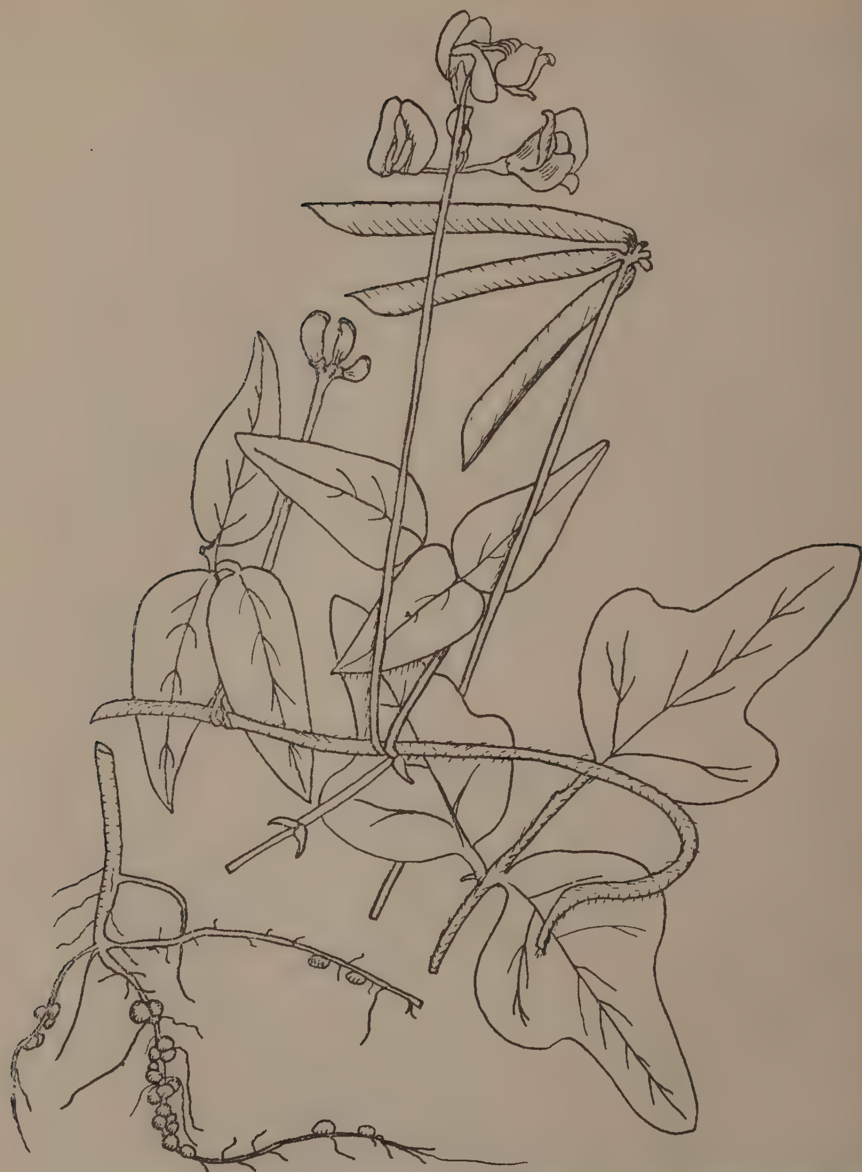

Fig. 1. A portion of a plant including a section of the vine with flowers, fruit, and leaves, and a portion of the root system bearing nodules.

Its seeds are rich in protein and starch (fig. 6). The seeds are shed soon after maturity and thus early become available for quails, pheasants, and other birds. I am not aware of any definite information on the use of the seeds by birds, but I have observed that quails frequent the areas where the plants grow and find, under or in the tangle of vines, which usually

persist through the winter, favorite roosting places. It reseeds readily and thus has the advantage of a perennial in the habit of self-propagation.

FRUITS

The pods, like the flowers, are borne in axillary clusters, which appear acropetally as the successive nodes develop. The pods are commonly two to three inches in length, almost cylindrical in shape, and contain four to eight bean-shaped seeds ranging from one-half to one centimeter in length. Soon after the seeds are mature the pods open by a splitting which is followed by a twisting of the valves that assists in the expulsion of the seeds. This habit of shedding the seeds soon after they are ripe is a disadvantage with reference to the harvesting of the seeds and especially so, since the pods ripen and open successively over a period of two to three months in length, but is an advantage in that the seeds are early available for birds.

EXTERNAL CHARACTERISTICS AND STRUCTURE OF THE SEEDS

The seeds have a flaky or hairy covering and range in color from grayish brown to black in the ripe dry condition, but change to a lighter color and become more polished in appearance as they imbibe water and swell preliminary to germination (figs. 1, 2 and 11). They are much elongated, their diameter usually ranging around one-half to one-third of their length, and slightly angular with a rib running the length of the back side and a short rib leading away from the chalazal end of the hilum. The hilum region is white or whitish, and is proportionately narrow for the size of the seed but unusually elongated, commonly being more than half the length of the seed (figs. 1 and 2). The hilum is considerably depressed and the depression is filled with a cottony tissue which is a remnant of the funiculus and responsible for the whiteness of the hilum region (figs. 1, 2 and 5). At one end of the hilum region the micropyle (m, fig. 2) is recognizable as a slit-like or circular depression, while at the other end there is a furrowed ridge (g, fig. 2) which, as shown in a cross section, marks a much thickened region in the chalazal portion of the seed coat (fig. 4). In a lengthwise median section of a seed, as shown in figure 3, the tip of the radicle (r) is found to lie very close to the pore-like opening (m) of the micropyle.

The flaky covering of grayish brown scales, characteristic of mature seed coats, led Pammel (3), Britton (4) and others to describe the seeds of *Strophostyles helvola* as having hairy seed coats. A study of the development of the seeds discloses that the flakes constituting the flaky covering of the seed coat, and generally designated as hairs, are the result of the sloughing in layers of the outer portion, known as the cuticularized layer, of the Malpighian cells (figs. 6, 7 and 8). This sloughing of the cuticularized layer accounts for the fact that the light line in mature seeds is exceptionally near to or on the surface of the seed coat, a feature noted by Pammel (7). The sloughing layers swell some in water and in case of seeds in the germinator favor the growth of molds.

The seed coat, aside from the exfoliation of the cuticularized layer, is typical of leguminous seed coats. It consists of Malpighian, osteosclerid and nutrient layers. The Malphighian layer is double in the region of the

hilum (fig. 5). The osteosclerid layer varies considerably in the character of its cells in different parts of the coat, but in general consists of cells with walls thickened in ridges and containing a brown pigment in mature seeds. The portion of the seed covering interior to the osteosclerid layer comprises two parts, the one just beneath the osteosclerized layer and consisting of several layers of thin-walled cells and an inner one that consists of a few layers of cells with walls somewhat thickened. The inner part is darkly colored and is in part responsible for the color of the seed. It separates rather readily in germination from the rest of the seed covering, appearing separately sometimes as a membrane over the embryo or portions of it after the rest of the covering is removed. This inner region contains the aleurone layer, which, according to Pammel (7), is endospermous in origin and all that remains of the endosperm in mature seeds. One striking feature of this inner part of the seed coat is that through it run tangentially many anastomosing tracheal elements (fig. 10). It contains no starch nor considerable amounts of any other forms of stored food.

The Malpighian layer, which abruptly bends inward at the margin of the hilum, forms a ridge that encloses a pit at the bottom of which is the hilum (fig. 5). Under the rim about the hilum and under the hilum, there is a mass of tissue that is continuous with the inner regions of the seed coat but much greater in thickness (fig. 5). In this thickened area and running lengthwise just beneath the mid region of the hilum is a ridge of tracheal-like elements known as the tracheal isle (v. fig. 5). Immediately over the ridge of the tracheal isle the Malpighian layers of the hilum are reduced in thickness or almost severed. The tracheal isle consists of cells which are elongated mainly at right angles to the hilum and have made their walls rigid by thickened lignified bands after the fashion of scalariform vessels, although it has not been shown that they have any connection with the regular vascular system through which the seed receives water and nutrients.

WINTERING AND GERMINATION OF SEEDS

Most of the seeds lie uncovered during the fall and winter beneath the plants from which they are shed and are thus available for birds when food is much needed. The seeds are prevented from germinating by the hard seed coats as shown by the fact that fully developed seeds while still green and seeds sufficiently scarified at any time after maturity give a high percentage of germination. Tests on seeds still green but fully developed gave a range of 40 to 70 per cent germination. Seeds with an opening pricked in the seed coat with a needle or in any other way germinate close to 100 per cent any time after maturity. Seeds caught by frost before entirely mature generally refuse to germinate on account of the condition of their embryo, although they may appear mature except for the fact that they have not been dehisced.

Germination tests on seeds brought from the field at various times during the winter and spring over a period of five years show that the seed coats of most of the seeds remain impermeable and delay germination till relatively late in the spring. In Story and Boone counties in central Iowa, where observations have been made on germination in the field, most of the germination occurs from the tenth of May to the first of June and consequently the plants generally escape destruction in the seedling stage by spring plowing. That the entrance of water is prevented by the

Malpighian layer and chiefly by its light line, is shown by the fact that after being scratched with a needle the seed coat is permeable. Also when the hard seeds are soaked in solutions of dyes, sections of their coats show that the dyes do not pass through the light line. Under natural conditions, as in the case of seeds that have become permeable through exposure to the conditions outdoors, the entrance of water is in the region of the hilum, usually at the microphyle where the radicle later emerges as if through a specially provided pore (fig. 9).

Fully mature seeds are quite generally hard, commonly germinating as low as five per cent. That the seeds require several months weathering to bring about permeability of the seed coats is shown by the following table.

TABLE 1. *Showing the percentage of germination in the laboratory of seeds brought from the field at different times during winter and spring. Five hundred seeds in each test. Germinated between blotters in moist chambers at room temperature*

	Percentage of germination				
Date of collection	1933-34	1934-35	1935-36	1936-37	Average
Nov. 1-Dec. 20	4	6	5	7	5.5
March 1-30	9	10	11	10	10.0
May 10-June 1	68	72	58	62	64

It is seen from table 1 that the seeds changed very little in permeability before May and observations in the field on germination showed that there was little germination prior to the tenth of May.

Seeds collected in the fall before frost and stored both dry and wet in a relatively constant temperature in the laboratory and refrigerator during the years the data were collected in the preceding table gave a low germination at the time the germination in the field was high. Seeds collected before frost in 1936, stored in laboratory until January 8, 1937, and then stored both wet and dry on an open porch showed no increase in germination as late as June 18. Seeds exposed alternately to laboratory and the freezing temperature of outdoors as many as 20 times during a period of 30 days showed practically no change in percentage of germination.

It is evident from the observations in the field and laboratory tests of the germination of seeds variously stored that a variation in temperature and probably also moisture over a period of several months at least is necessary to bring about the permeability of the seed coats. The variations over the period from the time of ripening to the middle of the following May is sufficient to open the coats of the majority of the seeds fully exposed but some require the exposure of at least a second year. The weather evidently works a change akin to deterioration in the seed coat, as shown by its becoming more brittle, and eventually bring about permeability.

After water enters through the seed coat it rapidly spreads around the embryo through the inner layers of the seed coat where the tracheal tubes are located. The seeds soon swell to double or more their dry dimensions, commonly become lighter in color and the seed coat tends to become smooth and glossy (fig. 11).

OPENING THE SEED COATS ARTIFICIALLY

The artificial methods used involved blows, pricking, and abrasions.

The effectiveness of blows was determined in two ways, namely by throwing the seeds back and forth in a quart Mason jar with as much force as could be done by hand, and by putting the seeds in a small muslin bag, one foot in length, and swinging the bag against the floor or table. By the last method the intensity of the blows must be modulated lest the seeds be broken into fragments and entirely destroyed. The number of seeds used in the determination ranged from 500 to 1,000.

The effectiveness of pricking was tested in two ways, by stabbing the seeds wtih a needle, and by throwing the seeds backward and forward by hand through a Mason jar against the surface of No. 2 sandpaper fitted in each end of the jar. The number of seeds used was 25 when pricking with the needle was employed, and 500 or more when the method of throwing the seeds against sandpaper was employed.

In the abrasion method the seeds were rubbed by hand between two sheets of No. 2 sandpaper for a few seconds. The seeds used in each case were collected in September and October and were stored in the laboratory.

The germination tests were made in moist chambers at room temperature. The results are given in table 2.

From the results given in table 2 it is obvious that pricking and abrad-

TABLE 2. *Showing the results of the various treatments in opening the seed coats*

Date seeds were collected	Type of treatment, date and percentage of germination									
	Thrown back and forth through a quart Mason jar		Beaten in a bag against the floor or table		Stabbed with a needle		Thrown back and forth through a Mason jar against sandpaper		Rubbed between sheets of sandpaper	
	Date	Pctg. Germ.	Date	Pctg. Germ.	Date	Pctg. Germ.	Date	Pctg. Germ.	Date	Pctg. Germ.
Sept. 1933	Dec. 8–20	12	Dec. 20–25	50 many bro-ken	Dec. 20–25	95	Jan. 1–10	76	Jan. 1–10	92
Sept. & Oct. 1935	Nov. 6–15	15	Nov. 6–15	52	Nov. 6–15	96	Nov. 6–15	68	Nov. 6–15	88
Sept. & Oct. 1936	Nov. & Dec. & Mch. 1937	16	Mch. 20–30 1937	57	Mch. 20–30 1937	95	Mch. 20–30 1937	60	Mch. 20–30 1937	93
Average		14		53		95		68		91

ing the seed coats are the most effective means of making the coats permeable. One would naturally expect this, since it is the light line that is impermeable and it is on or near the surface of the seed coat and thus exposed to pricking or abrasive agencies.

TYPES OF HABITAT

In most manuals, as Gray's (3), Britton's (1), Rydberg's (9) and Small's (10), the habitat of *Strophostyles helvola* is specifically stated to be sandy soil or sandy shores and river banks. In a catalogue of the flowering plants of Missouri by Palmer and Steyermark (8) its habitat is given as rocky woods and thickets. Harshberger (4) mentions it as one of the dominant plants on the boulder clay shores of Lake Ontario. In the Weed Flora of Iowa, Pammel (6) states that it is common in sandy places and along the chief rivers of the state. Although its habitat is prevailingly a sandy soil, it is not confined to shores and bottoms along streams, but is frequently found thriving on bluffs and hillsides where the soil is generally dry and contains only a small amount of sand, as in case of the Clarion fine sandy loam upon which the plant grows especially well in Iowa.

It is able apparently to tolerate considerable shading, as shown by the fact that it thrives in corn fields, grain fields, among weeds and in thickets. Soil acidity obviously is not so important in determining its distribution as it is in case of some legumes. Palmer and Steyermark (8) describe its habitat as circum-neutral. No definite data were found as to its tolerance of soil acidity, but samples of soil tested from a number of areas in Iowa where the plants were thrifty had a range of two and one-half tons per acre lime requirement to 7.5 pH. More soil tests very likely will show a greater range of tolerance in the pH of soils. Its range in tolerance of soil conditions is a feature worthy of note in considering it in relation to soil erosion.

HEAT AND DROUGHT RESISTANCE

The most striking characteristic of *Strophostyles helvola* is its heat and drought resistance. During the intense heat and drought of 1936, this plant made a good growth, flowered and set seed abundantly on the sandiest of soils and on southerly exposed bluffs, hillsides and eroded banks where both the heat and drought reached their maximum.

Its remarkable ability to endure drought and heat, its prolific habits and tolerance of shade and soil acidity, qualify it to be in the list of plants that may be of considerable value on eroded and sandy waste areas, either alone or among shrubs to furnish food and probably cover for birds. It is of value in improving the nitrogen content of the soil and may be of special value as a green manure. If it can be crossed with beans its drought and heat resistance may be combined with the more desirable features of some of the beans in crosses of more value for hay and seed than the *Strophostyles*.

SUMMARY

Strophostyles helvola (L.) Britton, an annual legume, is common throughout the Mississippi Valley and the states east to the Atlantic.

Its prevailing habitat is a sandy soil and consequently it is found much more generally in river bottoms and around lakes than elsewhere.

Its trailing vines sometimes interfere with cultivation, for which reason it may be classed as a weed.

It is a prolific plant, flowering and seeding from early July till frost, and propagating itself as effectively as many perennials.

The seeds are shed soon after maturity and thus early become available as food for birds. They are quite generally hard, germinating usually not more than 5 to 10 per cent without treatment.

The seeds have a hard, impermeable seed coat, that has a fuzzy covering which consists of the scales resulting from the sloughing of the layers composing the cuticularized portion of the Malpighian cells. The sloughing of these layers extends almost and frequently to the light line. The seeds are rich in protein and starch.

The light line prevents the entrance of water in hard seeds, and requires several months of exposure to the variations in fall, winter and spring weather to make it permeable. Most of the seeds fully exposed germinate between the tenth of May and the first of June of the following year, but some delay germination till the second year or longer.

The embryo requires no rest period, and seeds will germinate any time after maturity provided their coats are made permeable to water, which is rather easily accomplished by abrasive methods, such as rubbing the seeds between sheets of coarse sandpaper.

Strophostyles helvola is remarkably resistant to drought and heat, and tolerates a relatively wide range in the pH of soils. It has been observed thriving on bluffs and eroded hillsides where heat and drought were extreme. These traits. along with its prolific seeding and ability to propagate, qualify this plant for consideration with reference to its value on sandy wastes and eroding hillsides, either alone or with other plants, to furnish food and possibly cover for birds, and also to add nitrogen to the soil.

Owing to its close relationship to the true beans, there is the possibility that it may be used as a parent with some of the beans to obtain crosses of greater value than either of the parents for waste and eroded areas.

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PLATE I

EXPLANATION OF FIGURES

Fig. 1. Seeds, showing shape of seeds, the much elongated whitish hilum region, and the fuzziness of the seed coat.

Fig. 2. The hilum side of a seed showing micropyle (m) and the furrowed ridge (g) at the chalazal end of the hilum.

Fig. 3. Section through a seed showing the position of the tip of the radicle (r) in relation to the micropyle (m).

Fig. 4. Cross section of the furrowed ridge at the chalazal end of the hilum. Note the furrow in the apex of the ridge and the vertical strand of dark tissue beneath the furrow.

Fig. 5. Cross section of a seed through the hilum region, showing the depression, the white cotton-like tissue (c) filling the depression, the vascular isle (v), and that the Malpighian layer is double.

Fig. 6. Section through seed showing the cells of the cotyledons well filled with starch and protein.

Fig. 7. Section through seed coat showing especially the Malpighian cells (M), (the very much elongated cells that constitute more than half the thickness of the seed coat) and the several layers beneath of much smaller cells that constitute the osteosclerid and nutrient layers. Note the sloughing of the cuticularized layer (s), the fragments of which cause the fuzzy appearance of the seed coat.

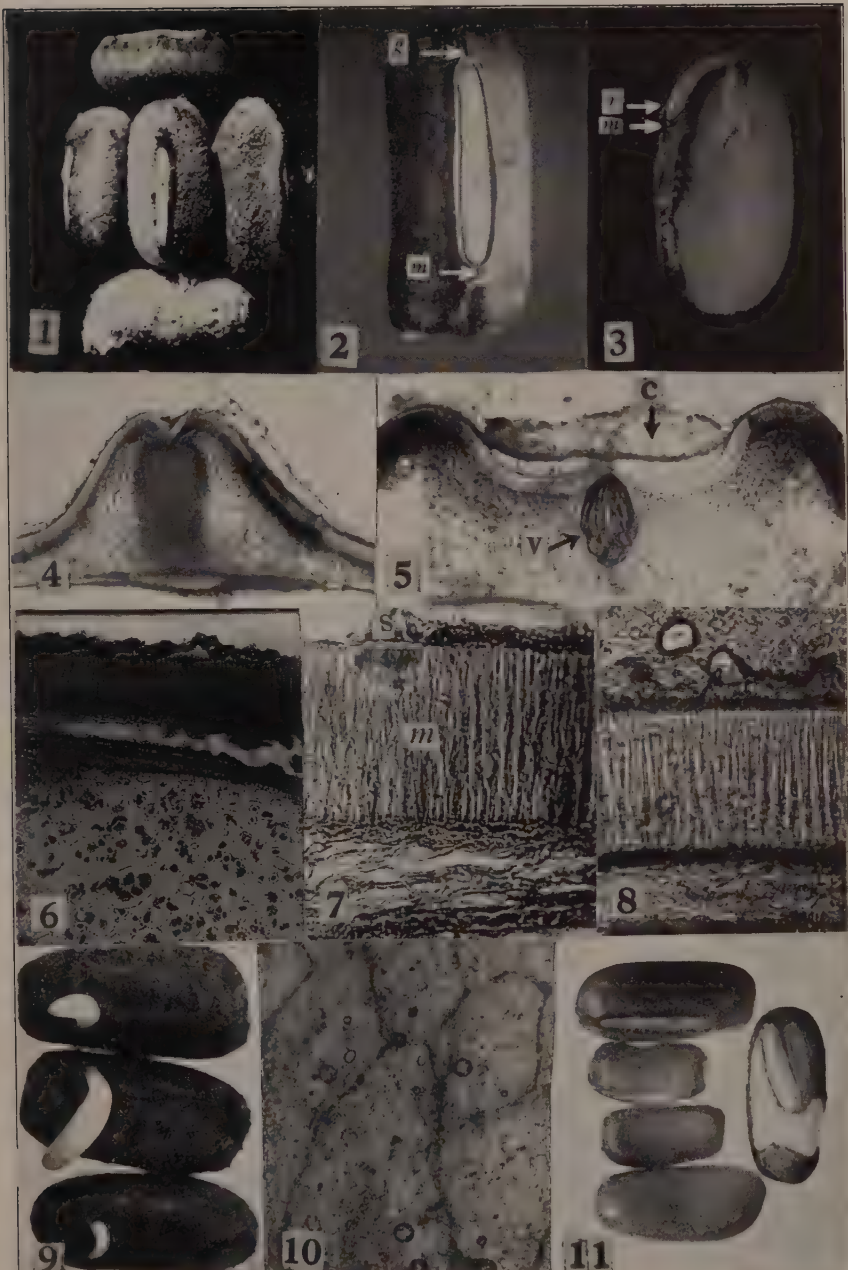
Fig. 8. A section similar to the one in figure 7, but showing more distinctly the light line just beneath the sloughing cuticularized layer which in this case has taken up water and become much swollen.

Fig. 9. Germinating seeds showing the radicles emerging at the micropyle.

Fig. 10. Surface view of the inner layer of the seed coat showing the vascular bundles.

Fig. 11. Seeds swollen preliminary to germination. Note polished appearance. The seed with the broken seed coat was scarified between sheets of sandpaper.

PLATE I



GROUND STATE OF THE LI ATOM[1]

CHARLES P. WELLS

From the Department of Mathematics, Iowa State College

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The allowed energy levels of a quantum mechanical system are the values of W that permit solutions of the Schrodinger wave equation

$$H\psi = W\psi$$

which are finite and continuous throughout space. Here H represents the Hamiltonian operator, and ψ the characteristic function. The solution of this wave equation corresponds to the solution of the variation problem (1)

$$\delta W = \delta \int \bar{\psi} H \psi \, d\tau = 0,$$

subject to the normalizing condition

$$\int \psi \bar{\psi} \, d\tau = 1.$$

The function ψ must, of course, satisfy all the conditions imposed on any solution of the wave equation. The direct method of solving the variation problem consists in selecting trial wave functions which contain parameters which are to be varied until W is a minimum. It can be shown that W is an upper limit to the energy and ψ is an approximate solution. The selection of the trial wave functions depends on the physical knowledge of the problem and experience with previous problems.

The method of forming correct anti-symmetric wave functions for many electron systems was developed by Heisenberg (2). Independently Wigner (3) showed how to form wave functions for lithium and three electron ions. Slater (4) has shown how to build up a properly anti-symmetric wave function for a many electron system by using a determinant whose elements are functions of single electrons only.

The best results on the value of the energy of the ground state of the lithium atom that have been published are those of Wilson (5), who used functions of the type

$$(K\text{-}shell)e^{-ar},\ (L\text{-}shell)\ a\, r\, e^{-\eta r} - e^{-\xi r}.$$

He used the variation method and was able to obtain a value of the energy within .86 per cent of the experimental. Previously Slater (6) and Guillemin and Zener (7) employing the same methods and simpler wave functions had obtained values within .88 per cent and .87 per cent of the experimental, respectively.

In all these papers the interaction of the electrons with each other is neglected in obtaining the solution. The purpose of this paper has been to

[1] A thesis submitted to the Graduate Faculty of Iowa State College in partial fulfillment of requirements for the degree of Doctor of Philosophy. Original thesis submitted August, 1935.

extend the work along the lines of Guillemin and Zener, to solutions of the wave equation containing the interaction of the inner electrons.

DISCUSSION OF THE METHOD

1. Wave Equation and Jacobian.
The wave equation for lithium is

$$\Delta_1\psi + \Delta_2\psi + \Delta_3\psi + \frac{8\pi^2\mu}{h^2}(W - V)\psi = 0, \tag{1}$$

where

$$V = -\frac{3e^2}{r_1} - \frac{3e^2}{r_2} - \frac{3e^2}{r_3} + \frac{e^2}{r_{12}} + \frac{e^2}{r_{13}} + \frac{e^2}{r_{23}}.$$

With the change of units

$$W = \frac{2\pi^2\mu e^4\lambda}{h^2},\ x_i = \frac{h^2 x'_i}{8\pi^2\mu e^2 Z}, i = 1, 2, 3, 4.$$

this becomes

$$\Delta_1\psi + \Delta_2\psi + \Delta_3\psi + \left[\frac{\lambda}{4} + \frac{1}{r_1} + \frac{1}{r_2} + \frac{1}{r_3} - \frac{1}{3r_{12}} - \frac{1}{3r_{23}} - \frac{1}{3r_{13}}\right]\psi = 0. \tag{2}$$

Hylleraas (8) has shown the advantage obtained by using as coordinates the r's entering into the potential energy V. The differential equation 1 is not self adjoint when written with the r's as coordinates. We can make it so by multiplication by the Jacobian of the transformation from rectangular coordinates to the six r's supplemented by three angular coordinates to make the system complete. These extra coordinates are ignorable and can be eliminated by integration. The Jacobian can be computed and found to be, aside from constant factors

$$J = \frac{r_1\ r_2\ r_3\ r_{12}\ r_{23}\ r_{13}}{A} \tag{3}$$

where

$$\begin{aligned} A^2 = {} & r_1^2 r_2^2 (r_{13}^2 + r_{23}^2 - r_{12}^2) + r_1^2 r_3^2 (r_{12}^2 + r_{23}^2 - r_{13}^2) \\ & + r_{12}^2 r_{23}^2 (r_1^2 + r_3^2) + r_2^2 r_3^2 (r_{12}^2 + r_{13}^2 - r_{23}^2) \\ & + r_{13}^2 r_{23}^2 (r_1^2 + r_2^2) + r_{12}^2 r_{13}^2 (r_2^2 + r_3^2) - r_2^2 r_{13}^2 (r_{13}^2 + r_2^2) \\ & - r_1^2 r_{23}^2 (r_1^2 + r_{23}^2) - r_3^2 r_{12}^2 (r_3^2 + r_{12}^2) - r_{13}^2 r_{23}^2 r_{12}^2. \end{aligned}$$

With this Jacobian as a factor, equation 1 now becomes the differential equation for a problem in the Calculus of Variations. This problem is the minimizing of the integral

$$\int J \left\{ \mid grad\, \psi \mid^2 + V\psi^2 \right\} d\tau = \lambda, \tag{4}$$

subject to

$$\tfrac{1}{4} \int J \psi^2 \, d\tau = 1.$$

2. Wave Functions Used.

With a notation similar to that of Guillemin and Zener (9) our wave function is

$$\psi = \frac{1}{\sqrt{6}} \sum_P (-1)^{\sigma_P} P \cdot \psi(x_{11}, x_{21}, x_{32}) \, \delta(\tfrac{1}{2}/m_{s_1}) \, \delta(-\tfrac{1}{2}/m_{s_2}) \, \delta(\tfrac{1}{2}/m_{s_3}), \quad (5)$$

where x_{ik} represents the i-th electron in the k-th orbit, P represents permutations of the three electrons, σ_P the number of inversions in the permutations and δ (m_s/m_{s_1}) designates the spin function of the i-th electrons with spin component m_s. The summation is over the six permutations.

The energy integral and the normalizing integral can be shown to reduce from thirty six terms each to two each. They are

$$\int J \psi_{123} H \psi_{123} \, d\tau - \int J \psi_{123} H \psi_{321} \, d\tau = \lambda,$$

$$\tfrac{1}{4} \left[\int \psi_{123} \psi_{123} \, d\tau - \int \psi_{123} \psi_{321} \, d\tau \right] = 1.$$

In these integrals and in what follows ψ_{123} and ψ_{321} represent $\psi(x_{11}, x_{21}, x_{32})$ and ψ (x_{31}, x_{21}, x_{12}) respectively.

The K-shell functions used are hydrogen like functions $e^{-(r_1+r_2)/2}$. The L-shell functions are an extension of Guillemin and Zener's. They are

$$e^{-ar_3/2} \left[1 + b\, r_3 + c\, r_{12} + d\, (r_1 - r_2)^3\right].$$

PRELIMINARIES TO CALCULATIONS

1. Definitions of M,L,N and $M';L',N'$.

As pointed out by Hylleraas (10), the use of a stretching factor for all the arguments in ψ i.e. ψ $(r) = \psi$ (kr), results in the minimum problem (2) becoming

$$\frac{K^2 M - K L}{N} = \text{min.} = \lambda \quad (6)$$

where

$$M = \int J \, |\, \text{grad}\, \psi \,|^2 \, d\tau, \; L = -\int J V \psi^2 \, d\tau, \; N = \tfrac{1}{4} \int J \psi^2 \, d\tau. \quad (7)$$

The parameter k can be eliminated by differentiation, obtaining for the minimum value of λ, the expression

$$-\frac{L^2}{4MN} = \lambda. \quad (8)$$

This expression will be a function of certain parameters, a,b,c,d, entering into the wave function. Their values can be found by solving a set of algebraic equations

$$\frac{2}{L}\frac{\partial L}{\partial a}-\frac{1}{M}\frac{\partial M}{\partial a}-\frac{1}{N}\frac{\partial N}{\partial a}=0, \tag{9}$$

etc, for all four parameters.

Our case is more general than that of Hylleraas due to the character of the function ψ. In place of equations 6 and 7 we have

$$\frac{K^2(M-M')-K(L-L')}{N-N'}=\lambda,$$

where

$$\begin{aligned} M &= \int J\,|\,grad\,\psi_{123}\,|^2\,d\,\tau, & M' &= \int J\,|\,grad\,\psi_{123}\cdot grad\,\psi_{321}\,|\,d\,\tau,\\ L &= -\int J\,V\,\psi^2_{123}\,d\,\tau, & L' &= \int J\,\psi_{123}\,V_{321}\,d\,\tau,\\ N &= -\int J\,\psi^2_{123}\,d\,\tau, & N' &= \int J\,\psi_{123}\,\psi_{321}\,d\,\tau. \end{aligned}$$

The parameter k can be eliminated as before by differentiation, obtaining for the new value of λ the expression

$$\frac{-(L-L')^2}{4(M-M')(N-N')}=\lambda. \tag{10}$$

2. Integrals Involved.

The integrals involved are all of the form

$$\int_0^\infty dr_1\int_0^\infty dr_2\int_0^\infty dr_3\int_{|r_1-r_2|}^{r_1+r_2} dr_{12}\int_{|r_2-r_3|}^{r_2+r_3} dr_{23}\int_p^q dr_{13}$$

$$\frac{r_1^m\,r_2^n\,r_3^p\,r_{12}^q\,r_{23}^s\,r_{13}^t}{\sqrt{(p^2-r_{13}^2)(r_{13}^2-p^2)}}\,e^{-\alpha r_1-\beta r_2-\gamma r_3} \tag{11}$$

The integrals in M and N are straightforward and cause no particular difficulty. Those in M' and N' can be carried out without essential difficulties, although they become long and tedious. Difficulty arises in L' because of the interaction term r_{12} in ψ. The product $\psi_{123}\,\psi_{321}$ carries the term $r_{12}\,r_{23}$, and the potential term $1/r_{13}$, thus giving rise to the integral

$$\int J\,\frac{r_{12}\,r_{23}}{r_{13}}\,d\,\tau \tag{12}$$

As easily seen, the first integration is elliptic and since the Jacobian is homogenous in the r's, changing the order of integration does not relieve the situation. However the integral can be approximated to a sufficient degree of accuracy by expanding $1/r_{13}$ in a series of Legendre Polynomials. Thus

$$1/r_{13} = 1/r_1 [P_0(\mu) + h\, P_1(\mu) + h^2\, P_2(\mu) + \ldots\ldots\ldots\ldots] \quad for\ h = r_3/r_1 < 1$$

and

$$1/r_{13} = 1/r_3\ [P_0(\mu) + h\, P_1(\mu) + h^2\, P_2(\mu) + \ldots\ldots\ldots\ldots] \quad for\ h = r_1/r_3 < 1$$

Substitution of these in (12) gives rise to two sets of integrals, one for $r_3 < r_1$ and the other for $r_1 < r_3$. The integrations can now all be carried out, but are exceedingly long and tedious. Use of three terms in the series gives a very good approximation for the integral.

CALCULATIONS AND RESULTS

The value of the parameters which make λ a minimum can be determined from (10) by differentiation. However the exponential parameter enters in such a complicated manner that it is not practicle to attempt differentiation. Instead the best value for this parameter is found by the use of the simpler trial functions, such as re^{-ar} and $e^{-ar}(1 + br)$. For both these functions the best value of a is found to be .26 and gives the value of the energy λ corresponding to these functions in error by only .88 and .87 per cent respectively. These agree with the values found by Slater and Guillemin and Zener. Using this is as a trial value for the parameter a, we substitute in the expressions for M- M', $L - L'$, and $N - N'$. The quadratic expressions in b, c and d that result are as follows:

$$M - M' = \pi(373.3064 + 9951.936b + 2028.898c + 2321.987d + 80{,}731.91b^2 + 5303.91c^2 + 73{,}195.97d^2 + 28{,}564.843bc + 17{,}840.19cd + 36{,}883.98bd)$$

$$L - L' = \pi(634.3433 + 17{,}458.17b + 4175.04c + 4132.497d + 144{,}010.97b^2 + 9557.235c^2 + 76{,}644.71d^2 + 61{,}204.87bc + 29{,}528.74cd + 68{,}769.28bd.)$$

$$N - N' = \pi(163.61 + 4643.48b + 1252.31c + 982.78d + 38{,}953.31b^2 + 3259.6c^2 + 18{,}850.78d^2 + 18{,}643.865bc + 7639.98cd + 16{,}762.54bd).$$

The best values for b, c and d can now be found by differentiation and are $b = -.29$, $c = -.13$, and $d = -.02$. The value of the energy calculated is $\lambda = -1.65967$. The experimental value is given at $\lambda = -1.663$ and the error is .2 per cent. Repeated calculations using a value of the parameter slightly greater and slightly less than $a = .26$ do not improve the value of λ.

The author wishes to take this opportunity to express his sincerest gratitude to Dr. J. V. Atanasoff for his direction and guidance in preparing this paper.

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MAMMALS OF IOWA

Thos. G. Scott

From the Department of Entomology and Zoology, Iowa State College[1]

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Revision of the mammalian faunal occurrence in Iowa is the primary objective of this investigation. The work involves an interpretation of former lists, perusal of mammal literature, examination of available material and field observations. It is to be hoped that this investigation will eventually contribute towards an interest in conserving the mammals, towards recognition of the systematist's value and towards assistance to specialists making detailed life history studies.

The preliminary work on this problem was commenced on October 1, 1936. A list of recent Iowa mammals was compiled from known distribution as offered by Miller (1924) and the various revisions in the North American Fauna series. Inclusions in this hypothetical list were then checked against a thorough perusal of the literature affecting Iowa mammals.

Determination of intra-state distribution and verification of questionable records made necessary the collection of representative specimens. During the course of the investigation, approximately 100 mammals suitable to identification were collected. The collections in which the included specimens repose are given mention in the text of the discussion. All of these specimens were examined by Dr. H. H. T. Jackson and A. H. Howell of the United States Biological Survey, and all final identifications are given on their authority. To facilitate further investigation and to verify doubtful records, an attempt was made to locate all museum specimens of Iowa mammals. Such specimens were found to repose in the collection of the United States Biological Survey, American Museum of Natural History, Field Museum of Natural History, Milwaukee Public Museum, Museum of Natural History at the University of Minnesota and the Museum of Zoology at the University of Michigan. There are also a few specimens in the museum at Coe College and at the University of Iowa and in various private collections; however, most of these, being life-like mounts, are not suitable to accurate identification. Even when considered in the aggregate, specimens of Iowa mammals are entirely too few to permit presentation of complete distributional data.

This list includes not only the 56 mammals known to occur in Iowa today, but also those which have been exterminated through land use. Of the latter, the American Black Bear, Canada Otter, Rocky Mountain Cougar, Canada Lynx, Wildcat, American Elk and Plains Bison are known to have been represented. Others, such as the American Marten, Fisher, Common Wolverine, Swift Fox, Black Rat, Canada Porcupine and American Pronghorn, are thought to have been present, but no suitable evidence for their occurrence has been uncovered. These mammals and a few which ought to occur in the state but for which no authentic proof is available are included in a hypothetical supplement. Eleven forms which have appeared in former lists through misinterpretation of the known

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[1] Iowa State College, Iowa Conservation Commission, and American Wildlife Institute cooperating with the United States Bureau of Biological Survey.

range or description are contained in a separate group accompanied by an explanation for their removal.

The nomenclature and systematic sequence are those presented in the "List of North American Recent Mammals, 1923," by Gerrit S. Miller, Jr., 1924, except where revision has been made. The common names are those created by H. E. Anthony in his "Field Book of North American Malmals," 1928. Each mammal is introduced by the common name. This is followed by the current scientific name and a brief synonymy. The synonymy commences with the publication of the original description and the type locality, and concludes with the names used by Allen (1871), Goding (1883), Osborn (1890), Van Hyning and Pellett (1910), Ruthven and Wood (1912), Stoner (1918), and Gabrielson (1921).

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Special acknowledgment is made to the late Professor J. E. Guthrie for a small but valuable collection of specimens and for early guidance in the study of mammalogy.

REVIEW OF THE LITERATURE

The review is limited chiefly to former lists of Iowa mammals; however, recognition is given the reports of early surveys enfolding occasional notes on natural history. The earliest of these is volume 59 of the "Jesuit Relations," monumental report of Marquette and Joliet. In 1673, these men passed along the eastern boundary of Iowa on the Mississippi River from the mouth of the Wisconsin River southward.

Lewis and Clark were the first to report on Western Iowa, ascending the Missouri River in 1804 and returning by way of the same route in 1806. Thomas Say, member of Major Long's expedition, remained through the winter of 1819-20 at Engineer Cantonment near the present town of Blair, in Washington County, Nebraska. Description of Say's *Canis nubilus, Canis latrans Sorex brevicaudus* = *Blarina b. breicauda* and *Sorex parvus* = *Cryptotis parva* as contained in volume one of "Long's Expedition to the Rocky Mountains" is from specimens taken during the stay at Engineer Cantonment.

Several expeditions through Iowa were headed by Stephen Watts Kearny, officer in the United States Army. Kearny led an expedition from the vicinity of Council Bluffs to the junction of the Minnesota and Mississippi Rivers and southward on the Father of Waters to St. Louis. Brigadier-General Henry Atkinson accompanied Kearny on a two-year exploration of the Missouri River, starting from St. Louis in 1824. In 1835, Kearny made his last journey over Iowa. This time he went from Keokuk northwest to Boonesboro, northeast to Lake Pepin, Minnesota, and returned over the same route. The eighth number of volume 12 in the Palimpsest contains a summarization of Kearny's travels in Iowa by W. J. Peterson.

Prince Maximilian of Wied, preparator of the earliest list of Indiana mammals, passed up the Missouri River in 1833 and returned in 1834. Wied's "Travels in the Interior of North America" was translated from the German and published by the Arthur H. Clark Company of Cleveland, Ohio, in 1906.

Probably the first attempt at enumeration of Iowa mammals was made by Dr. Isaac Galland of Montrose, Lee County, in 1840. "Galland's Iowa Emigrant" appears to be a description of the natural resources of Iowa Territory. The mammals are discussed under the subheading, "Beasts." Since Dr. Galland settled in Montrose in 1827 and died there in 1858, one may expect his writing to be reflective of Iowa. A reprint of Galland's book may be found in the Annals of Iowa, 1921, pp. 481-509.

A review of John J. Audubon's visits to Iowa during his trip up the Missouri River in 1843 is given in volume 5 of the Annals of Iowa.

Dr. J. A. Allen (1871) wrote the first list of Iowa mammals. In 1867, Allen spent the months of July, August and September making scientific observations on the wildlife of Iowa. Short visits were paid Boonesboro, Boone County, and Denison, Crawford County, but the investigation was carried on largely in Dallas, Guthrie, Greene, Carroll, Sac, Calhoun and Audubon counties. The 48 species listed by Allen are included from observations during the investigation or from known distribution. It is of signficance that Allen fails to give recognition to *Cynonomys ludovicianus ludovicianus, Martes pennanti pennanti, Martes americana americana, Gulo luscus, Spilogale interrupta, Lynx canadensis canadensis, Lepus townsendii campanius* and *Antilocapra americana americana.* Allen's list remains as the most scientific preparation of its kind for Iowa mammals.

In 1882, Dr. W. F. Goding (1883) presented a list of the wild and domesticated mammals of Iowa at the meetings of the Iowa Agricultural Society. This work appears to have been based almost entirely upon interpretation of the literature, and is therefore a somewhat questionable reference.

The third list of Iowa mammals was offered by Dr. Herbert Osborn during the session of the Iowa Academy of Science in 1888. The paper was published in the proceedings of that organization in 1890. Osborn (1890, p. 41) reveals that he was in knowledge of Goding's work, since he writes: "I must add that a very few of the species included in Dr. Goding's list seem to me extremely doubtful." The species bearing doubtful notation were: *Martes pennanti pennanti, Condylura cristata, Gulo luscus, Peromyscus nuttali aureolus, Ochetodon humilis = Reithrodontomys h. humilis* and *Lepus callotis.* "A Partial Catalogue of the Animals of Iowa" by Osborn was published by the Iowa Agricultural College in 1892. The mammals catalogued were, with some exceptions, supposed to have been represented by mounted specimens in the college museum. Evidence indicates that specimens were considered representative of Iowa mammals regardless of the locality record. A narrative dealing with the extinct and vanishing animals of the state is given by Osborn in the Annals of Iowa (1905).

The Proceedings of the Iowa Academy of Science for 1910 contain a catalogue of Iowa mammals prepared by Van Hyning and Pellett. These writers exhibit a lack of familiarity with the literature and fail to include reference to museum specimens. Of the 88 mammals presented in this list,

33 are given a hypothetical rating. This paper by Van Hyning and Pellett represents the last attempt to write a complete list of Iowa mammals.

An authentic record of the mammals of Clay and Palo Alto counties was prepared by Ruthven and Wood (1912). The record is based upon specimens collected in the vicinity from 1907 to 1912 and upon observations made during the expedition of 1907. The specimens are contained in the Museum of Zoology at the University of Michigan.

"Rodents of Iowa," by Dayton Stoner (1918), provides valuable information on this large group of mammals. Helpful contributions are made by Spurrell (1917) for Sac County, by Gabrielson (1921) for Marshall County, and by Stephens (1922) for the lake region of Dickinson County.

Literature on the mammals of Iowa is not abundant. Perhaps it is because the mammals, with few exceptions, fail in appeal to the esthetic sense of man and are not subject to easy observation.

CLASSIFIED LIST

MAMMALS OF KNOWN RECENT OCCURRENCE

CLASS MAMMALIA

Order 1. Marsupialia

Family Didelphiidae

Virginia Opossum, *Didelphis virginiana virginiana* Kerr.

Order 2. Insectivora

Family Talpidae

Prairie Mole, *Scalopus aquaticus machrinus* (Rafinesque).
Missouri Valley Mole, *Scalopus aquaticus machrinoides* Jackson.

Family Soricidae

Masked Shrew, *Sorex cinereus cinereus* I. Geoffroy.
Hayden Shrew, *Sorex cinereus haydeni* (Baird).
Hoy Pigmy Shrew, *Microsorex hoyi hoyi* (Baird).
Little Short-tailed Shrew, *Cryptotis parva* (Say).
Large Short-tailed Shrew, *Blarina brevicauda brevicauda* (Say).

Order 3. Chiroptera

Family Vespertilionidae

Little Brown Bat, *Myotis lucifugus lucifugus* (Le Conte).
Trouessart's Bat, *Myotis keenii septentrionalis* (Trouessart).
Silver-haired Bat, *Lasionycteris noctivagans* (Le Conte).
Big Brown Bat, *Eptesicus fuscus fuscus* (Beauvois).
Northern Red Bat, *Nycteris borealis borealis* (Müller).
Hoary Bat, *Nycteris cinerea* (Beauvois).

Family Molossidae

Tacubaya Free-tailed Bat, *Tadarida depressa* (Ward).

Order 4. Carnivora

Family Ursidae

American Black Bear, *Euarctos americanus americanus* (Pallas).

Family Procyonidae

Eastern Raccoon, *Procyon lotor lotor* (Linnaeus).

Family Mustelidae

Bonaparte Weasel, *Mustela cicognani cicognani* Bonaparte.
Minnesota Weasel, *Mustela longicauda spadix* (Bangs).
Common Mink, *Mustela vison mink* (Peale and Beauvois).
Mississippi Valley Mink, *Mustela vison letifera* Hollister.
Canada Otter, *Lutra canadensis canadensis* (Schreber).
Prairie Spotted Skunk, *Spilogale interrupta* (Rafinesque).
Northern Plains Skunk, *Mephitis hudsonica* (Richardson).
Illinois Skunk, *Mephitis mesomelas avia* (Bangs).
Common Badger, *Taxidea taxus taxus* (Schreber).

Family Canidae

Northern Plains Red Fox, *Vulpes regalis* Merriam.
Wisconsin Gray Fox, *Urocyon cinereoargenteus ocythous* Bangs.
Northern Coyote, *Canis latrans* Say.
Timber Wolf, *Canis nubilus* Say.

Family Felidae

Rocky Mountain Cougar, *Felis oregonensis hippolestes* (Merriam).
Canada Lynx, *Lynx canadensis canadensis* Kerr.
Wildcat, *Lynx rufus rufus* (Schreber).

Order 5. Rodentia

Family Sciuridae

Southern Woodchuck, *Marmota monax monax* (Linnaeus).
Thirteen-striped Ground Squirrel, *Citellus tridecemlineatus tridecemlineatus* (Mitchill).
Franklin Ground Squirrel, *Citellus franklini* (Sabine).
Gray Eastern Chipmunk, *Tamias striatus griseus* Mearns.
Southern Red Squirrel, *Sciurus hudsonicus loquax* Bangs.
Minnesota Red Squirrel, *Sciurus hudsonicus minnesota* Allen.
Northern Gray Squirrel, *Sciurus carolinensis leucotis* (Gapper).
Western Fox Squirrel, *Sciurus niger rufiventer* (Geoffroy).
Small Eastern Flying Squirrel, *Glaucomys volans volans* (Linnaeus).

Family Geomyidae

Shaw Pocket Gopher, *Geomys bursarius* (Shaw).

Family Heteromyidae

Dusky Pocket Mouse, *Perognathus flavescens perniger* Osgood.

Family Castoridae

Missouri River Beaver, *Castor canadensis missouriensis* Bailey.

Family Cricetidae

Short-eared Grasshopper Mouse, *Onychomys leucogaster breviauritus* Hollister.
Prairie Harvest Mouse, *Reithrodontomys megalotis dychei* (Allen).
Baird White-footed Mouse, *Peromyscus maniculatus bairdi* (Hoy and Kennicott).
Northern White-footed Mouse, *Peromyscus leucopus noveboracensis* (Fischer).
Goss Lemming Mouse, *Synaptomys cooperi gossii* (Coues).
Pennsylvania Meadow Mouse, *Microtus pennsylvanicus pennsylvanicus* (Ord).
Prairie Meadow Mouse, *Microtus ochrogaster* (Wagner).
Woodland Pine Mouse, *Pitymys nemoralis* (Bailey).
Common Muskrat, *Ondatra zibethica zibethica* (Linnaeus).
Great Plains Muskrat, *Ondatra zibethica cinnamomina* (Hollister).

Family Muridae

House Mouse, *Mus musculus musculus* Linnaeus.
Norway Rat, *Rattus norvegicus* (Erxleben).

Family Zapodidae

Prairie Jumping Mouse, *Zapus hudsonius campestris* Preble.

Order 6. Lagomorpha
Family Leporidae

White-tailed Jack Rabbit, *Lepus townsendii campanius* Hollister.
Mearns Cottontail, *Sylvilagus floridanus mearnsi* (Allen).

Order 7. Artiodactyla
Family Cervidae

American Elk, *Cervus canadensis canadensis* (Erxleben).
Plains White-tailed Deer, *Odocoileus virginianus macrourus* (Rafinesque).

Family Bovidae

Plains Bison, *Bison bison bison* (Linnaeus).

MAMMALS OF HYPOTHETICAL OCCURRENCE

Order 3. Chiroptera
Family Vespertilionidae

Little Gray Bat, *Myotis griscens* Howell.
Georgian Bat, *Pipistrellus subflavus subflavus* (F. Cuvier).
Rafinesque Bat, *Nycticeius humeralis* (Rafinesque).

Order 4. Carnivora
Family Mustelidae

American Marten, *Martes americana americana* (Turton).

Fisher, *Martes pennanti pennanti* (Erxleben).
New York Weasel, *Mustela noveboracensis noveboracensis* (Emmons).
Common Wolverine, *Gulo luscus* (Linnaeus).
Long-tailed Texas Skunk, *Mephitis mesomelas varians* (Gray).

Family CANIDAE

Swift Fox, *Vulpes velox velox* (Say).

Order 5. RODENTIA

Family SCIURIDAE

Rufescent Woodchuck, *Marmota monax refescens* Howell.
Southern Gray Squirrel, *Sciurus carolinensis carolinensis* Gmelin.

Family CASTORIDAE

Canadian Beaver, *Castor canadensis canadensis* Kuhl.

Family CRICETIDAE

Little Gray Harvest Mouse, *Reithrodontomys albescens griseus* (Bailey).

Family MURIDAE

Black Rat, *Rattus rattus rattus* (Linnaeus).

Family ZAPODIDAE

Hudson Bay Jumping Mouse, *Zapus hudsonius hudsonius* (Zimmermann).

Family ERETHIZONTIDAE

Canada Porcupine, *Erethizon dorsatum dorsatum* (Linnaeus).

Order 7. ARTIODACTYLA

Family ANTILOCAPRIDAE

American Pronghorn, *Antilocapra americana americana* (Ord).

MISINTERPRETATIONS

Order 2. INSECTIVORA

Family TALPIDAE

Star-nosed Mole, *Condylura cristata* (Linnaeus).

Order 4. CARNIVORA

Family CANIDAE

Eastern Red Fox, *Vulpes fulva* (Desmarest).

Order 5. RODENTIA

Family SCIURIDAE

Black-tailed Prairie Dog, *Cynomys ludovicianus ludovicianus* (Ord).
Eastern Chipmunk, *Tamias striatus striatus* (Linnaeus).

Family Cricetidae

Southern Golden Mouse, *Peromyscus nuttalli aureolus* (Audubon and Bachman).
Bailey Wood Rat, *Neotoma floridana baileyi* (Merriam).
Drummond Meadow Mouse, *Microtus drummondi* (Audubon and Bachman).

Order 6. Lagomorpha

Family Leporidae

Minnesota Varying Hare, *Lepus americanus phaeonotus* Allen.
Wagler's Jack Rabbit, *Lepus callotis* Wagler.
Great Plains Jack Rabbit, *Lepus californicus melanotis* (Mearns).

Order 7. Artiodactyla

Family Cervidae

Common Moose, *Alces americana americana* (Clinton).

THE MAMMALS OF IOWA

Order 1. Marsupialia

Family Didelphiidae

VIRGINIA OPOSSUM
Didelphis virginiana virginiana Kerr

1792. *Didelphis virginiana* Kerr, Anim. Kingd., p. 193.
Type Locality: Virginia.
1871. *Didelphys virginiana* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 194.
1883. *Didelphys virginiana* Goding, Iowa State Agr. Soc. (1882), p. 331.
1890. *Diadelphys* (sic) *virginiana* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 44.
1910. *Didelphis virginiana* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 212.
1912. *Didelphis virginiana* Ruthven and Wood, Proc. Iowa Acad. Sci., vol. 19, p. 203.
1921. *Didelphis virginiana* Gabrielson, Proc. Iowa Acad. Sci., vol. 28, p. 147.

The Virginia Opossum, typically Carolinean, is a relatively new addition to the mammalian fauna of Iowa. It may be found along timbered watercourses throughout the state, somewhat common in the southern five tiers of counties and progressively less numerous toward the north. The northern limits of distribution have been extended most rapidly along the streams, especially those of the Mississippi and Missouri River valleys. The species is still considered rare in the north central counties.

The opossum was probably present in Iowa long before the arrival of the first settlers. It was included in a discussion of the natural resources of the Territory of Iowa by Dr. Izaac Galland (1840), and early settlers in Sac County told of seeing opossums at Grant and Lee groves upon their arrival in 1854 (Spurrell, 1917). An intensive faunal investigation of Dallas, Guthrie, Boone, Greene, Carroll, Crawford, Sac, Calhoun, and Audubon counties, by Dr. J. A. Allen during the summer of 1867 failed to afford the opportunity of collecting or observing this species; however, it was listed upon authentic report, as rare in southern Iowa (Allen, 1871). It is readily seen that Allen's investigation was carried out

high on the watershed between the Missouri and Mississippi Rivers, and therefore on the remote end of the wooded avenues of approach along streams. Perhaps that is why the opossum did not appear in that region until a later date.

The species under discussion first appeared at Atlantic, Cass County, some time during the '90's, according to Frank C. Pellett (letter, 1936). Seton (1929, 4: 870) quotes Senator Lafayette Young as stating in 1915: "Opossums are now common as far up as Des Moines. It first came about 10 years ago." Residents in the vicinity of Marshalltown were unfamiliar with this mammal in 1914 (Gabrielson, 1921). Spurrell (1917) tells of seeing two opossums taken in traps at Wall Lake, Sac County, in 1907, at which time they were just beginning to move out from the large streams. It is listed for Clay County by Ruthven and Wood (1912) on an authentic record obtained for Gillett's Grove. This record indicates that the opossum was just appearing in the region by way of the Little Sioux River. Beyond the timbered lanes of our streams and their tributaries, the advance of the opossum has been retarded. Stephens (1922) gives the opossum only a hypothetical rating for Dickinson County. The writer has made two significant observations in relation to opossums in northern Iowa. Remains of an opossum were found in the stomach of a red fox taken at Ryan Lake, Emmet County, during the 1936-1937 winter, and an opossum, victim of highway traffic, was seen on state highway 13, about two miles south of Elkader, Clayton County, on August 16, 1936.

Order 2. Insectivora

Family Talpidae

PRAIRIE MOLE

Scalopus aquaticus machrinus (Rafinesque)

1832. *Talpa machrina* Rafinesque, Atlantic Journal, vol. 1, p. 61. Type Locality: Near Lexington, Fayette County, Kentucky.

1883. *Scalops argentatus* Goding, Iowa State Agr. Soc. (1882), p. 330.

1890. *Scalops argentatus* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42.

1910. *Scalops aquaticus machrinus* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 215.

1921. *Scalopus aquaticus machrinus* Gabrielson, Proc. Iowa Acad. Sci., vol. 28, p. 149.

The Prairie Mole is found wherever food and soil are suitable throughout eastern Iowa.

The original range of this mole in Iowa, according to a map by Jackson (1915), is everywhere east of a line from Keokuk northwest along the Des Moines River to Knoxville and due north to Deercreek on the Iowa-Minnesota state line. This range may now be extended about 30 miles to the west. The old line marking the western limits for this mole in Iowa is changed only between Knoxville and Deercreek, where it reaches out to inculde Jewell, Hamilton County. This change is made on the basis of a specimen, No. 18a, taken at Jewell on March 8, 1936. Identification of this specimen was verified by H. H. T. Jackson of the United States Biological Survey. The specimen is now in the Iowa State College collection. Surber (1932) reports that *machrinus* has not been taken in Minnesota.

This subspecies is listed for Dickinson County by Stephens (1922) and for Sac County by Spurrell (1917). Both of these records are far be-

yond the known range and are not supported by specimens; hence, these records are misidentifications and undoubtedly refer to Jackson's *S. a. machrinoides.*

There are four specimens of this mole from Fairport, Muscatine County, in the private collection of Thaddeus Surber, Superintendent of Fish Propagation, Minnesota Department of Conservation (letter, 1936). George G. Goodwin, Assistant Curator of the American Museum of Natural History, writes that there are seven specimens from Iowa City, Johnson County, in the museum collection (letter, 1936).

Published records of museum specimens: Marshall Co., Marshalltown, Gabrielson (1921), 1, probably in the collection of the United States Biological Survey; Marion Co., Knoxville, Jackson (1915), 1, Field Museum of Natural History; Henry Co., Hillsboro, Jackson (1915), 1, collection of the United States Biological Survey.

Specimens examined:

Hamilton County, Jewell, No. 18a, Iowa State College collection.

MISSOURI VALLEY MOLE
Scalopus aquaticus machrinoides Jackson

1914. *Scalopus aquaticus machrinoides* Jackson, Proc. Biol. Soc. Washington, vol. 27, p. 19. Type Locality: Manhattan, Riley County, Kansas.

1871. *Scalops argentatus* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 187. (Not Rafinesque.)

1890. *Scalops aquaticus* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42. (Not Linnaeus.)

The Missouri Valley Mole is found wherever food and soil are suitable throughout western Iowa.

The range of this subspecies in Iowa is everywhere west of a line almost due north and south from Missouri to Minnesota through Des Moines (Jackson, 1915). It has been reported east as far as the Mississippi River from both Minnesota (Surber, 1932) and Missouri (Jackosn (1915); hence, increased collection should give reason for extending the eastern limits of this mole's range in Iowa. No point of intergradation has been established within the state.

Allen (1871) lists *Scalops argentatus* = *S. a. machrinus* as well known but not numerous. The investigations of Dr. Allen were all well within the known range of the more recently described *machrinoides.* In addition to *Scalops argentatus* = *S. a. machrinus,* Osborn (1890) lists *Scalops aquaticus* = *S. a. aquaticus* which is definitely a misidentification. It is possible that Osborn noticed a difference in Iowa moles and made reference to the only descriptions available at the time, in which case the reference may be considered a misidentification of *machrinoides.* Osborn appears to have considered this record a mistake, for it is not contained in a second list (Osborn, 1892). No explanation for this omission in the second list is given.

Published records of museum specimens: Pottawattamie Co., Council Bluffs, Jackson (1915), 1, collection of the United States Biological Survey.

Specimens examined:

Story County, Ames, No. 22a, Iowa State College collection.

Family SORICIDAE

MASKED SHREW

Sorex cinereus cinereus I. Geoffroy.

1792. *Sorex arcticus cinereus* Kerr, Animal Kingdom, p. 206. Type Locality: Fort Severn, Ontario, Canada.
1871. *Sorex cooperi* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 187.
1883. *Sorex cooperi,* Goding, Iowa State Agr. Soc. (1882), p. 330.
1890. *Sorex cooperi* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.
1910. *Sorex personatus* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 215.

The Masked Shrew is common in moist habitats supporting rank vegetative growth throughout northeast Iowa. The known range for this shrew in Iowa, as interpreted from a map by Jackson (1929), appears to include Mitchell, Howard, Winneshiek, Allamakee, Floyd, Chickasaw, Fayette, Clayton, Bremer, Black Hawk, Buchanan, Delaware and Dubuque counties. Nothing has been uncovered to provide reason for material alteration of the ascertained range. It may be assumed that the range extends westward to Hamilton County, on grounds of a specimen, No. 14a, taken on the edge of a marsh at Jewell. The specimen is referable to *S. c. haydeni* but exhibits a tendency to approach *S. c. cinereus.* The identification was verified by Dr. H. H. T. Jackson.

Sorex cooperi = *S. c. cinereus* was listed for Iowa on known distribution by Allen (1871). Osborn (1890) offers the notation, "fairly common;" Stephens (1922) includes *Sorex personatus* = *S. c. cinereus* on hypothetical grounds for the lake regions in Dickinson County. The reference is assumed to be a misidentification of Baird's *haydeni.*

Published records of museum specimens: Buchanan Co., Jackson (1928), 2, collection of the United States Biological Survey.

HAYDEN SHREW

Sorex cinereus haydeni (Baird).

1857. *Sorex haydeni* Baird, Report Pacific R. R. Survey 8: part 1, Mammals, p. 29. Type Locality: Fort Union, Nebraska (later Fort Buford, now Mondak, Mont., near Buford, Williams County, N. Dak.).

The Hayden Shrew is common in moist areas supporting rank growths of vegetation throughout northwest Iowa.

The known distribution, interpreted from a map by Jackson (1928), apparently includes Lyon, Osceola, Dickinson, Sioux, O'Brien, Clay, Plymouth, Cherokee, Buena Vista, Woodbury, Ida and Sac counties. Specimens taken during this investigation provide reason for extending the range of this subspecies in Iowa. A specimen, No. 2a, was collected at Arnold's Park, Dickinson County, and two more, No. 7a and No. 14a, were taken in a *Scirpus-Typha* associes at Jewell, Hamilton County. These new records permit the original range in Iowa to be extended eastward to a line drawn from Spirit Lake, Dickinson County, southeastward to Jewell, Hamilton County, and thence due west through Wall Lake, Sac County, to the Missouri River. It may be assumed that the true margin of this shrew's present range is located near Jewell, Hamilton County. This assumption is based on a specimen, No. 14a, which has been mentioned be-

fore as showing an approach to *S. c. cinereus.* According to Jackson (1928, p. 52) such a characteristic might serve to indicate the margin of the range, since he writes: "It is a rather variable form particularly in cranial characters, which show everywhere in a broad border along its range an approach toward *S. c. cinereus.*"

The first record of this shrew in Iowa is given by Spurrell (1917) for Sac County. The specimens mentioned by Spurrell are recorded by Jackson (1926).

Published records of museum specimens: Sac Co., Wall Lake, 1, and Sac City, 1, Jackson (1928), collection of the United States Biological Survey.

Specimens examined:

Hamilton County, Jewell; Nos. 7a and 14a; Iowa State College collection.

Dickinson County, Arnold's Park; No. 2a; Iowa State College collection.

HOY PIGMY SHREW
Microsorex hoyi hoyi (Baird).

1857. *Sorex hoyi* Baird, Report Pacific R. R. Survey, vol. 8, part 1, Mammals, p. 32. Type Locality: Racine, Racine County, Wisconsin.

The probable range of the Hoy Pigmy Shrew in Iowa, according to a map presented by Jackson (1928), is over northeast Iowa, north of a line drawn from the vicinity of Dubuque northwestward to Spirit Lake. Jackson had no specimens from Iowa, but assumed that the range passed through this part of Iowa from specimens taken in Minnesota, South Dakota and Wisconsin.

Specimen, No. 21a, representing the first record of this shrew for Iowa, was taken at Clear Lake, Cerro Gordo County, during these investigations. The specimen was referred to this subspecies by Dr. H. H. T. Jackson.

Specimens examined:

Cerro Gordo County, Clear Lake; No. 21a; Iowa State College collection.

LITTLE SHORT-TAILED SHREW
Cryptotis parva (Say).

1823. *Sorex parvus* Say, Long's Exped. Rocky Mts., vol. 1, p. 163. Type Locality: West bank of Missouri River, near Blair, Washington County, Nebraska.

1883. *Blarina exilipes* Goding, Iowa State Agr. Soc. (1882), p. 330.

1890. *Blarina exilipes* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.

1910. *Blarina parva* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 215.

1921. *Cryptotis parva parva* (sic) Gabrielson, Proc. Iowa Acad. Sci., vol. 28, p. 149.

The Little Short-tailed Shrew is found throughout Iowa. The known range, as given by Miller (1924), includes all Iowa. This species has been included on state lists by Goding (1883), Osborn (1890 and 1892), and Van Hyning and Pellett (1910). Van Hyning and Pellett (1910, p. 215) offer the following notation: "Common over the state."

It is recorded for Marshall County by Gabrielson (1921) and for Sac County by Spurrell (1917). The writer has examined specimens from Marion and Story counties, both of which were too badly crushed to permit preparation.

There are two specimens of this shrew from Fairport, Muscatine County, in the private collection of Thaddeus Surber, Superintendent of Fish Propagation, Minnesota Department of Conservation (letter, 1936).

LARGE SHORT-TAILED SHREW
Blarina brevicauda brevicauda (Say).

1823. *Sorex brevicaudus* Say, Long's Exped. Rocky Mts., vol. 1, p. 164.
Type Locality: West bank of Missouri River, near Blair, Washington County, Nebraska.

1871. *Blarina brevicauda* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 187.

1883. *Blarina brevicauda* Goding, Iowa State Agr. Soc. (1882), p. 330.

1890. *Blarina brevicauda* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.

1910. *Blarina brevicauda* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 215.

1921. *Blarina brevicauda brevicauda* Gabrielson, Proc. Iowa Acad. Sci., vol. 28, p. 149.

The Large Short-tailed Shrew is found throughout all Iowa, more numerous in the wooded parts than in the open fields.

The writer has chosen to follow Anthony (1928), Surber (1932) and others in considering Gapper's subspecies *talpoides* as indistinguishable from *brevicauda*.

There are three specimens of this shrew from Fairport, Muscatine County, in the private collection of Thaddeus Surber, Superintendent of Fish Propagation, Minnesota Department of Conservation (letter, 1936). One specimen from Iowa City, Johnson County, is contained in the American Museum of Natural History (Goodwin, letter, 1936), and another from Knoxville, Marion County, is contained in the Field Museum of Natural History (Simms, letter, 1936).

Published records of museum specimens: Dickinson Co., Stephens (1922), 1, private collection; Pottawattamie Co., Council Bluffs, Merriam (1895), 8, collection of the United States Biological Survey; Marion Co., Knoxville, Merriam (1895), 2, collection of the United States Biological Survey.

Specimens examined:

Story County, Ames; Nos. 51a, 144, 121, and 135; Iowa State College collection.

Hamilton County, Jewell; Nos. 3a and 11a; Iowa State College collection.

Order 3. Chiroptera
Family Vespertilionidae

LITTLE BROWN BAT
Myotis lucifugus lucifugus (Le Conte).

1831. *Vespertilio lucifugus* Le Conte, McMurtrie's Cuvier, Animal Kingdom, vol. 1, p. 431.
Type Locality: Georgia.

1890. *Vespertilio lucifugus* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42.
1910. *Myotis lucifugus* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 215.

The Little Brown Bat is found throughout all Iowa. Migratory habits cause it to be irregularly numerous in a given locality from season to season.

Surber (1932) considers this subspecies as the most common bat in Minnesota. It is recorded for Nebraska as "Uncommon eastwardly" (Swenk, 1915, p. 854).

Authentic records have been made for National, Clayton County, by Sherman (1929) and for Dickinson County by Stephens (1922).

There is a specimen of this bat from Fairport, Muscatine County, in the private collection of Thaddeus Surber, Superintendent of Fish Propagation, Minnesota Department of Conservation (letter, 1936).

TROUESSART'S BAT
Myotis keenii septentrionalis (Trouessart)

1897. *Vespertilio gryphus* var. *septentrionalis* Trouessart, Catal. Mamm. VIV. foss., p. 131.
Type Locality: Halifax, Nova Scotia.
1871. *Vespertilio subulatus* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 187 (Not Say).
1883. *Vespertillio* (sic) *subulatus* Goding, Iowa State Agr. Soc. (1882), p. 330 (Not Say).
1890. *Vespertilio subulatus* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42 (Not Say).
1910. *Myotis subulatus* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 215 (Not Say).

The status of Trouessart's Bat is undetermined for Iowa. Lyon (1936, p. 65) writes that "Trouessart's Bat is distributed in eastern North America from Newfoundland and Quebec, south to Tennessee and South Carolina, west to North Dakota and Arkansas."

In addition to inclusion by the writers recognized in the synonymy, this bat is listed by Osborn (1892). The latter record is supposed to have been supported by a specimen in the collection of the Iowa State College. The specimen cannot be found, and the value of the specimen as an Iowa record is somewhat doubtful (see p. 9). To the writer's knowledge, there are no authentic published records for museum specimens from Iowa.

Van Hyning and Pellett (1910) list this species as common throughout the state. It is considered "Of but local distribution in Minnesota, . . ." by Surber (1932, p. 45).

SILVER-HAIRED BAT
Lasionycteris noctivagans (Le Conte)

1831. *V* [*espertilio*] *noctivagans* Le Conte, McMurtrie's Cuvier, Animal Kingdom, vol. 1, p. 431.
Type Locality: Eastern United States.
1871. *Scotophilus noctivagans* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 187.
1883. *Vespertillo noctivigans* (sic) Goding, Iowa State Agr. Soc. (1882), p. 330.
1890. *Vesperugo noctivigans* (sic) Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42.
1910. *Lasionycteris noctivagans* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 215.

The Silver-Haired Bat occurs irregularly along the wooded streams in Iowa. It migrates southward upon the approach of autumn, and at that time may be seen flying during the day.

The known range is "North America north of Mexico, from the Atlantic to the Pacific; probably not breeding south of the transition zone" (Miller, 1924, p. 74). For Nebraska, Swenk (1915, p. 854) writes that the species is "Common over the state in migrations." It is believed to be widely distributed in Minnesota (Surber, 1932).

To the writer's knowledge, there are no authentic published records for museum specimens from Iowa.

BIG BROWN BAT
Eptesicus fuscus fuscus (Beauvois)

1796. *Vespertillio fuscus* Beauvois, Catl. Raisonne Mus. Peale, Philadelphia, p. 18.
Type Locality: Philadelphia, Pennsylvania.
1871. *Scotophilus fuscus* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 187.
1890. *Vesperugo serotina* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42.
1910. *Vespertilio fuscus* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 215.

The Big Brown Bat is found throughout all Iowa. It has been observed at National, Clayton County, by Sherman (1929). Mr. Fred J. Pierce reports the species from Delaware County (letter, 1937).

George C. Goodwin, Assistant Curator, writes that a specimen of this bat from Iowa City, Johnson County, is contained in the American Museum of Natural History (letter, 1936). Two specimens from Dubuque, Dubuque County, are to be found in the Field Museum of Natural History, according to Director Simms (letter, 1936).

NORTHERN RED BAT
Lasiurus borealis borealis (Müller)

1776. *Vespertilio borealis* Müller, Natursyst. Suppl. p. 20.
Type Locality: New York.
1871. *Lasiurus noveboracensis* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 187.
1890. *Atalpha noveboracensis* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42.
1919. *Lasiurus borealis* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 215.
1921. *Nycteris borealis borealis* Gabrielson, Proc. Iowa Acad. Sci., vol. 28, p. 149.

The Northern Red Bat is found throughout the timbered parts of Iowa. It is considered our most abundant bat by Osborn (1890). Swenk (1915, p. 854) notes that this species is "Common over the state, especially eastwardly" in Nebraska. In Minnesota it is almost as common as the Little Brown Bat (Surber, 1932).

Authentic records are at hand from Marshall County by Gabrielson (1921) and for Sac County by Spurrell (1917). There is a female with two young from Ames, Story County, on display in the Department of Zoology and Entomology at Iowa State College.

Specimens examined:

Story County, Ames: 3, Iowa State College collection.

HOARY BAT
Lasiurus cinereus (Beauvois)

1796. *Vespertilio cinereus* (misspelled linereus) Beauvois, Catl. Raisonnè Mus. Peale, Philadelphia, p. 18.
Type Locality: Philadelphia, Pennsylvania.
1871. *Lasiurus cinereus* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 187.
1883. *Vespertillio* (sic) *pruinosus* Goding, Iowa State Agr. Soc. (1882), p. 330.
1890. *Atalpha cinereus* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42.
1910. *Lasiurus cinereus* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 216.
1912. *Lasiurus cinereus* Ruthven and Wood, Proc. Iowa Acad. Sci., vol. 19, p. 205.

The Hoary Bat is probably most common as a spring and fall migrant in Iowa. Swenk (1915, p. 854) considers this bat a "Very common migrant over the state." It is thought to be rare in Minnesota (Surber, 1932). The known range is: "Boreal North America from Atlantic to Pacific, breeding within the Boreal Zone, but in autumn and winter migrating at least to southern border of United States" (Miller, 1897, p. 112). The mounted specimen in the Iowa State College Museum was taken July 2, 1901.

Authentic records have been made for Palo Alto County by Ruthven and Wood (1912), for Dickinson County by Stephens (1922), and for Sac County by Spurrell (1917).

Specimens examined:

Story County, Ames: 1, Iowa State College Museum.

Family Mollossidae

TACUBAYA FREE-TAILED BAT
Tadarida depressa (Ward)

1891. *Nyctinomus depressus* Ward, Amer. Nat., vol. 25, p. 747.
Type Locality: Tacubaya, Federal District, Mexico.
1921. *Nyctinomus depressus* Gabrielson, Proc. Iowa Acad. Sci., vol. 28, p. 149.

The Tacubaya Free-Tailed Bat appears as a straggler in southern Iowa.

Published records of museum specimens: Marshall Co., Marshalltown, Gabrielson (1921), 1, Field Museum of Natural History (Simms, letter, 1936); Linn Co., Cedar Rapids, Cory (1912), 1, location of specimen not given.

Order 4. Carnivora

Family Ursidae

AMERICAN BLACK BEAR
Euarctos americanus americanus (Pallas)

1780. *Ursus americanus* Pallas, Spicilegia zoologica, fasc. 14, p. 5.
Type Locality: Eastern North America.
1871. *Ursus arctos* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 184.
1883. *Ursus Americanus* (sic) Goding, Iowa State Agr. Soc. (1882), p. 330.
1890. *Ursus arctos* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42.
1910. *Ursus americanus* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 217.

The American Black Bear is extinct in Iowa. It was common throughout the wooded parts of the state until about 1850.

A bear was killed in the vicinity of the junction of Chequest Creek and the Des Moines River in 1838, according to Duffield (1903, p. 209), who writes: "Hanging near one of the tepees was the carcass of a large bear, the skin still on, and none of the meat seemed to have been taken out." In 1840 Galland (1921, p. 500) writes: "Bears are scarce, but the Indians succeed every winter in obtaining more or less of these animals, as appears from the skins which they bring to the traders." An early settler reported a bear for Sac County in 1855 (Spurrell, 1917).

Cory (1912) reports the bear to be extinct in Illinois and gives the latest record as that of a bear taken in Alexander County during the year 1860. The black bear is still somewhat common in Minnesota as far south as the central part of Pine County (Surber, 1932).

Family Procyonidae

EASTERN RACCOON
Procyon lotor lotor (Linnaeus)

1758. [*Ursus*] *lotor* Linnaeus, Syst. Nat., ed. 10, vol. 1, p. 48.
Type Locality: Eastern United States.
1871. *Procyon lotor* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 184.
1883. *Procyon lotor* Goding, Iowa State Agr. Soc. (1882), p. 330.
1890. *Procyon lotor* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42.
1910. *Procyon lotor* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 216.
1921. *Procyon lotor lotor* Gabrielson, Proc. Iowa Acad. Sci., vol. 28, p. 149.

The Eastern Raccoon is found along the wooded streams throughout the state. This mammal is believed common; however, its numbers continue to be limited by the destruction of den trees.

Specimens examined:

"Iowa": 3, Iowa State College Museum.

BONAPARTE WEASEL
Mustela cicognani cicognani Bonaparte

1838. *M* [*ustela*] *cigognanii* (sic) Bonaparte, Charlesworth's Mag. Nat. Hist., vol. 2, p. 37.
Type Locality: Northeastern North America.
1871. *Putorius vulgaris* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 183.
1883. *Putorius pusillus* Goding, Iowa State Agr. Soc. (1882), p. 330.
1890. *Putorius vulgaris* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42.

The only authentic record of *cicognani* in Iowa is based on a specimen taken in the lake region of Dickinson County (Stephens, 1922). This specimen was referred to *Mustela c. cicognani* by Dr. H. H. T. Jackson.

MINNESOTA WEASEL
Mustela longicauda spadix (Bangs)

1896. *Putorius longicauda spadix* Bangs, Proc. Biol. Soc. Washington, vol. 10, p. 8.
Type Locality: Fort Snelling, Hennepin County, Minnesota.
1871. *Putorius ermineus* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 183.
1883. *Putorius ermineus* Goding, Iowa State Agr. Soc. (1882), p. 330.
1890. *Putorius ermineus* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42.

1910. *Putorius longicauda* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 217.

1912. *Putorius longicaudus* (sic) Ruthven and Wood, Proc. Iowa Acad. Sci., vol. 19, p. 205.

1921. *Putorius longicauda* subsp. Gabrielson, Proc. Iowa Acad. Sci., vol. 28, p. 149.

The Minnesota Weasel is more or less common throughout the state.

Authentic records are given for Johnson County by Nutting (1892), for Palo Alto County by Ruthven and Wood (1912), for Marshall County by Gabrielson (1921), for Dickinson County by Stephens (1922), and for Sac County by Spurrell (1917).

George G. Goodwin, Assistant Curator, writes that a specimen of this subspecies from Webb, in Clay County, is contained in the American Museum of Natural History (letter, 1936).

Published records of specimens: Johnson Co., Nutting (1892), 1, University of Iowa Museum; Palo Alto Co., Ruthven and Wood (1912), 1, Museum of Zoology at the University of Michigan; Sac Co., Spurrell (1917), 2, Smith collection at Sac City.

COMMON MINK
Mustela vison mink (Peale and Beauvois)

1796. *Mustela mink* Peale and Beauvois, Catl. Peale's Mus., Philadelphia, p. 39. Type Locality: Maryland.

1871. *Putorius lutreolus* Allen, Proc. Boston. Soc. Nat. Hist., vol. 13, p. 183.

1883. *Putorius lutreolus* Goding, Iowa State Agr. Soc. (1882), p. 330.

1890. *Putorius lutreolus* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42.

1910. *Lutreola vison* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 217.

1921. *Putorius vison lutreocephalus* Gabrielson, Proc. Iowa Acad. Sci., vol 28, p. 149.

The status of the Common Mink in Iowa is undetermined. This subspecies probably ranges throughout the state, increasing in numbers towards the south.

A specimen of this subspecies from Iowa City, in Johnson County, is contained in the American Museum of Natural History (Goodwin, letter, 1936). This is the only definite record of this mink in Iowa.

MISSISSIPPI VALLEY MINK
Mustela vison letifora Hollister

1913. *Mustela vison letifora* Hollister, Proc. U. S. Nat. Mus., vol. 44, p. 475. Type Locality: Elk River, Sherburne County, Minnesota.

The status of the Mississippi Valley Mink is undetermined for Iowa. The range probably extends throughout the state, increasing in numbers toward the north. Surber (1932) gives the range of this subspecies in Minnesota to the Iowa border.

The only published record for *letifora* in Iowa is offered by Spurrell (1917), who bases the record on a specimen in the Smith collection.

Specimens examined:

Decatur County, Leon: Nos. 39a, 40a and 47a (skulls); Iowa State College collection.

Boone County, Luther: No. 36a; Iowa State College collection

(All of the specimens in the Iowa State College collection were examined by A. H. Howell of the United States Biological Survey.)

CANADA OTTER
Lutra canadensis canadensis (Schreber)

1776. *Lutra canadensis* Schreber, Saugethiere, pl. 126b.
Type Locality: Eastern Canada.
1871. *Lutra canadensis* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 183.
1883. *Lutra canadensis* Goding, Iowa State Agr. Soc. (1882), p. 330.
1890. *Lutra canadensis* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42.
1910. *Lutra canadensis* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 217.

The Canada Otter was formerly found throughout the state wherever a supply of food in the form of fish was to be found. Through persistent trapping the otter became uncommon at an early date. Galland (1921) observed that the animal was becoming scarce. The reports received by Allen (1871, p. 183) during the summer of 1867 led him to write: "Said to be common on the Raccoon rivers, and generally more or less so throughout the State." The otter is considered rare by Osborn (1890), and was thought to have been etxinct about 1890 by Van Hyning and Pellett (1910).

The otter is well-known as a wanderer and continued to appear at irregular intervals throughout the state until about 1913. The specimen contained in the Iowa State College Museum was taken at Cambridge, in Story County, in the spring of 1881.

Van Hyning (1913, p. 312) quotes from a letter by George H. Berry, of Cedar Rapids, April 8, 1913: "Two otter went up the Cedar River on the ice in December and were tracked in the snow for nearly eight miles. Dr. Bailey of Coe College is negotiating for the skin of one caught the past winter, near Albia." Van Hyning (1913, p. 311) fails to name the writer of a letter composed at Knoxville, in Marion County, February 24, 1913: "M. W. Conwell, a local furrier, displays the skin of a large otter recently trapped on the Des Moines River, near Harvey, ten miles east of Knoxville. The pelt is in fine condition from the standpoint of the furrier, and is 5 feet 9 inches from tip to tip. The animal was trapped by John Morgan. About a week ago one of Mr. Morgan's traps was sprung by an otter which gnawed its leg off and escaped." This information probably came from G. K. Cherrie, who collected mammals in the vicinity of Knoxville, especially during the last of the nineteenth century. The latter information probably represents the last published record of the occurrence of the otter in Iowa.

Specimen examined:

Story County, Cambridge: 1, Iowa State College Museum.

PRAIRIE SPOTTED SKUNK
Spilogale interrupta (Rafinesque)

1820. *Mephitis interrupta* Rafinesque, Annals of Nature, vol. 1, p. 3.
Type Locality: Upper Missouri.
1910. *Spilogale interrupta* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 217.
1912. *Spilogale interrupta* Ruthven and Wood, Proc. Iowa Acad. Sci., vol. 19, p. 205.
1921. *Spilogale interrupta* Gabrielson, Proc. Iowa Acad. Sci., vol. 28, p. 149.

The Prairie Spotted Skunk is a southern species which has extended its range over Iowa in recent years.

The first authentic record of this skunk's presence in Iowa was made at Grinnell in 1870 by Parker (1870). Spurrell (1917) writes that early settlers in Sac County reported trapping the first spotted skunk in 1858. It was obviously scarce in the state as late as 1892, since it is not listed by such early writers as Allen (1871), Goding (1883) and Osborn (1890 and 1892). It was so scarce that Nutting (1892), unaware of Parker's publication, records this species as "New to the State" from specimens taken at Iowa City, in Johnson County. Spurrell (1917) writes that this species became numerous in Sac County in 1890. In 1902, trappers around Marshalltown, in Marshall County, told Seton (1929, vol. 2) that this species was new and increasing. A fresh specimen of this species taken February 3, 1902, at Marshalltown, in Marshall County, is sketched by Seton (1929, 2: 395). By 1907 it was considered quite common in Clay and Palo Alto counties by Ruthven and Wood (1912).

George G. Goodwin, Assistant Curator, writes that there are seven specimens of this species from Iowa City, Johnson County, contained in the American Museum of Natural History (letter, 1936).

Published records of specimens: Tama Co., Gladbrook, A. H. Howell (1906), 1, collection of United States Biological Survey; Marshall Co., Marshalltown, A. H. Howell (1906), 2, collection of United States Biological Survey.

Specimens examined:

Decatur County, Leon; No. 42a (skull); Iowa State College collection.

NORTHERN PLAINS SKUNK
Mephitis hudsonica (Richardson)

1829. *Mephitis americana* var. *hudsonica* Richardson, Fauna Boreali-Americana, vol. 1, p. 55.

Type Locality: Plains of Saskatchewan, Canada.

1910. *Chincha hudsonica* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 217.

1912. *Chincha hudsonica* Ruthven and Wood, Proc. Iowa Acad. Sci., vol. 19, p. 204.

The Northern Plains Skunk is found throughout the northern one-half of the state. The southern limit of the range has not been determined, but it is probably through central Iowa.

The only authentic published record for this species in Iowa is by Ruthven and Wood (1912). This record is based on two specimens taken during the expedition of 1907 in Clay and Palo Alto counties. The specimens are in the Museum of Zoology at the University of Michigan.

Mephitis hudsonica is found throughout Minnesota (Surber, 1932), but it is not listed for Missouri (Bennitt and Nagel, 1937).

ILLINOIS SKUNK
Mephitis mesomelas avia (Bangs)

1898. *Mephitis avia* Bangs, Proc. Biol. Soc. Washington, vol. 12, p. 32.
Type Locality: San Jose, Mason County, Illinois.

1871. *Mephitis mephitica* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 183.

1883. *Mephitis mephitica* Goding, Iowa State Agr. Soc. (1882), p. 330.

1890. *Mephitis mephitica* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42.
1910. *Chincha mesomeles* (sic) *avia* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 217.
1921. *Mephitis mephitis* (sic) *avia* Gabrielson, Proc. Iowa Acad Sci., vol. 28, p. 148.

The Illinois Skunk is found in southern Iowa with the northern limit of its range undetermined. The northward extension of its range is probably greater in the east than it is in the west. It is found throughout Missouri by Bennitt and Nagel (1937)), but there is no record for Minnesota.

Spurrell (1917) lists *Mephitis mesomelas avia* for Sac County, but the reference is open to question. An authentic record of *avia* is based on a skull taken at Ames and referred to this subspecies by A. H. Howell of the United States Biological Survey.

Published records of museum specimens: Delaware Co., A. H. Howell (1901), 1, collection of the United States Biological Survey.

Specimens examined:

Story County, Ames; No. 38a (skull); Iowa State College collection.

COMMON BADGER
Taxidea taxus taxus (Schreber)

1778. *Ursus taxus* Schreber, Saugthiere, vol. 3, p. 520
Type Locality: Labrador and Hudson Bay.
1871. *Taxidea americana* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 183.
1883. *Taxidea Americana* (sic) Goding, Iowa State Agr. Soc. (1882), p. 330.
1890. *Taxidea americana* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42.
1910. *Taxidea americana* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 217.
1912. *Taxidea taxus* Ruthven and Wood, Proc. Iowa Acad. Sci., vol. 19, p. 204.

The Common Badger is found in all Iowa. Badger populations exhibit a fluctuation, the cause of which would make an interesting and valuable determination.

In 1883, it was recorded as rare (Goding, 1883); in 1905 Osborn (1905) believed that there were few if any remaining, and by 1910 it was thought to be extinct (Van Hyning and Pellett, 1910). It was later found not to have become extinct (Van Hyning, 1913).

Opinion indicates that this mammal has shown a recent increase in numbers. Trapping records obtained from biennial and unpublished reports of the Iowa Conservation Commission appear to bear this out.

Season	Number taken	Average value
1930-31	75	Undetermined (Unpublished report, 1931)
1931-32	56	Undetermined (Unpublished report, 1932)
1932-33	17	\$4.00 (Iowa, 1934, p. 27)
1933-34	227	\$3.75 (Iowa, 1934, p. 27)
1934-35	207	\$5.50 (Iowa, 1936, p. 37)
1935-36	611	\$5.12 (Iowa, 1936, p. 37)

Specimens examined:

"S. E. Iowa"; museum No. 1; Iowa State College Museum.

Family CANIDAE

NORTHERN PLAINS RED FOX
Vulpes regalis Merriam

1900. *Vulpes regalis,* Merriam, Proc. Washington Acad. Sci., vol. 2, p. 672.
Type Locality: Elk River, Sherburne Co., Minnesota.

1871. *Vulpes vulgaris* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 192 (Not Desmarest).

1883. *Vulpes vulgaris* Goding, Iowa State Agr. Soc. (1882), p. 330 (Not Desmarest).

1890. *Vulpes vulgaris* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42 (Not Desmarest).

1910. *Vulpes pennsylvanicus* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 217 (Not Desmarest).

1921. *Vulpes fulvus fulvus* Gabrielson, Proc. Iowa Acad. Sci., vol. 28, p. 148 (Not Desmarest).

The Northern Plains Red Fox is found throughout all Iowa, being most numerous over its ancient range in the northern part of the state.

All published records of the red fox in Iowa refer to *fulva*. Only one record can be so construed as to appear to have been substantiated by a museum skin. C. C. Nutting (1895, p. 43) refers to this specimen, taken in Johnson County, as "The red fox, *Vulpes velox* . . ." and represents it as being in the Unviersity of Iowa Museum. Nutting explains that this fox was becoming more numerous in southeastern Iowa at the time, 1894; hence, there is no doubt but that the correct reference is to *regalis* and not to *velox*, a plains species with no authentic standing in Iowa. Nutting's specimen is not in the University of Iowa Museum (Dill, letter, 1937).

In 1867, the red fox was numerous from Wall Lake northward and occasional south of that point (Allen, 1871). The original range was gradually extended southward. Pellett writes that it first appeared at Atlantic, in Cass County, during the 1900's (letter, 1936). Despite persistent hunting and year around open season, the red fox continues to maintain its population throughout the state and to increase its numbers in the extended range over southern Iowa.

During this investigation specimens were taken at Estherville, in Emmet County, at Missouri Valley, in Harrison County, and at Leon, in Decatur County. All of these specimens have been referred to *regalis* by A. H. Howell.

Specimens examined:

Emmet County, Estherville: No. 45a (skull); Iowa State College collection.

Harrison County, Missouri Valley: No. 47a (skin and skull); Iowa State College collection.

Decatur County, Leon: Nos. 48a, 49a, 50a, 52a, 53a, 54a, 55a, 56a, 57a, 58a, 59a, and 60a (skulls); Iowa State College and United States Biological Survey collections.

WISCONSIN GRAY FOX
Urocyon cinereoargenteus ocythus Bangs

1899. *Urocyon cinereoargenteus ocythus* Bangs, Proc. New England Zool. Club, vol. 1, p. 43.
Type Locality: Platteville, Grant County, Wisconsin.

1871. *Vulpes virginianus* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 182.

1883. *Vulpes virginianus* Goding, Iowa State Agr. Soc. (1882), p. 330.
1890. *Urocyon virginianus* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42.
1910. *Urocyon cinereoargenteus* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 218.

The Wisconsin Gray Fox is found over northeastern Iowa. The limits of the range have not been determined.

The gray fox was believed "Frequent, but not especially numerous" by Allen (1871, p. 43). It is listed for Sac County by Spurrell (1917) on questionable report. Its presence there is extremely doubtful.

Specimens examined:

"Iowa"; museum No. 1; Iowa State College Museum.

NORTHERN COYOTE
Canis latrans Say

1823. *Canis latrans* Say, Long's exped. Rocky Mts., vol. 1, p. 168.
Type Locality: Blair, Washington County, Nebraska.
1871. *Canis latrans* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 181.
1883. *Canis latrans* Goding, Iowa State Agr. Soc. (1882), p. 329.
1890. *Chrysocyon latrans* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42.
1910. *Canis latrans* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 218.
1912. *Canis latrans* Ruthven and Wood, Proc. Iowa Acad. Sci., vol. 19, p. 204.
1921. *Canis latrans latrans* (sic) Gabrielson, Proc. Iowa Acad. Sci., vol. 28, p. 148.

The Northern Coyote appears to maintain occasional and irregular appearance throughout the state, showing a natural inclination for the less settled areas. As early as 1867 it was considered as formerly numerous (Allen, 1871). The Coyote has been given a more or less occasional rating by the more recent writers.

During this investigation specimens were taken at Missouri Valley, in Harrison County, and at Leon, in Decatur County. These specimens were referred to *latrans* by A. H. Howell of the United States Biological Survey.

Published records of museum specimens: Linn Co., Cory (1912), 2 skulls, Coe College collection.

Specimens examined:

Decatur County, Leon; No. 46a (skull); Iowa State College collection.

Harrison County, Missouri Valley; No. 44a (skull); Iowa State College collection.

TIMBER WOLF
Canis nubilus Say

1823. *Canis nubilus* Say, Long's Exped. Rocky Mts., vol. 1, p. 169.
Type Locality: Blair, Washington County, Nebraska.
1871. *Canis lupus* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 181.
1883. *Canis occidentalis* Goding, Iowa State Agr. Soc. (1882), p. 329.
1890. *Canis lupus* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 41.
1910. *Canis nubilus* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 218.

The Timber Wolf is of irregular occurrence throughout the state, appearing most frequently in the southeastern counties.

Allen (1871, p. 181) indicates that this species was common until about the middle of the last century, since writes: ". . . common less than 20 years since, they are now scarce . . ." In the 1890's, the wolf was thought to be increasing in northern Iowa (Nutting, 1893 and 1895). Spurrell (1917), writing for Sac County, received the information that the last wolf had been killed in 1868. Mr. P. Johann, Conservation Officer, reported two wolves as passing through Emmet County during the winter of 1935-36. Newspaper articles in respect to wolves appear from time to time, but a satisfactory tracing has not been effected as yet. Frequently such references are to *latrans*.

The recent survey of Bennitt and Nagel (1937, p. 26) in Missouri found *nubilus* to be "Common to rare in all counties, more numerous in southern Missouri."

Family Felidae

ROCKY MOUNTAIN COUGAR
Felis oregonensis hippolestes (Merriam)

1897. *Felis hippolestes* Merriam, Proc. Biol. Soc. Washington, vol. II, p. 219.
Type Locality: Wind River Mountains, Fremont County, Wyoming.
1871. *Felis concolor* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 181.
1883. *Felis concolor*, Goding, Iowa Agr. Soc. (1882), p. 329.
1890. *Felis concolor* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 41.
1910. *Felis concolor* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 218.

The Rocky Mountain Cougar formerly appeared throughout the wooded parts of the state.

The species is included in all the former lists of Iowa mammals from Allen (1871) to Van Hyning and Pellett (1910). These records are made largely upon known distribution and report. In 1840 Galland (1921, p. 500) writes: "The Panther is rarely seen in the country; their skins are to be found among the Indians, but I have not seen the animal alive in the country." A rather colorful report is offered by Van Hyning (1913, p. 312), but he fails to give the source of information. He quotes: "After a furious battle this morning (April 13, 1909) with a mountain lion, which sprung upon him while he was hunting on an island in Rush Lake, Walter Strauss of this place (Ocheyedan, Osceola County) finally killed the animal with a well directed shot from his Winchester. The animal weighed 160 pounds—measuring six feet from the nose to tip of tail." Swenk (1915, p. 853) writes for Nebraska: "Formerly common, now rare or extinct northwestwardly." It was reported to have been seen in Illinois as late as 1905 (Cory, 1912).

CANADA LYNX
Lynx canadensis canadensis Kerr

1792. *Lynx canadensis* Kerr, Anim. Kingd., vol. 1, systematic catalogue inserted between pages 32 and 33 (description, p. 157).
Type Locality: Eastern Canada.
1883. *Lynx canadensis* Goding, Iowa State Agr. Soc. (1882), p. 329.
1890. *Lynx canadensis* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 44.
1910. *Lynx canadensis*, Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 218.

The Canada Lynx entered northern Iowa as a straggler before and during the early days of settlement. The species has been included in all state lists since Goding (1883). It is found in an early list of the mammals for Cook County, Illinois (Kennicott, 1855).

Swenk (1915, p. 353), writing for Nebraska, considers it "Very rare northwardly, probably etxinct in the state." Records of *canadensis*, as made by untrained observers, are almost inseparable from those for *rufus*. Spurrell (1917) provides one of the more accurate records, since he was informed by professional trappers in Sac County of three taken in 1869 and one in 1875. On the authority of Spurrell's inclusion, it is believed safe to accept the statement of Mosher (1882), who writes: "A few Canada Lynxes are here, but they are rare; four have been killed here since I came." There is a record of this lynx being taken on the island south of Muscatine (Van Hyning, 1913). The specimen is said to have been the size of a wolf. A small wolf is at least twice the weight of the largest lynx. Increased doubt is found for the record since it is so far off the natural range. The specimen, which was supposed to have been prepared by a taxidermist at Iowa City, has not been located.

WILDCAT

Lynx rufus rufus (Schreber)

1777. *Felis rufa* Schreber, Saughthiere, pl. 109b.
    Type Locality: New York.
1871. *Lynx rufus* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 181.
1883. *Lynx rufus* Goding, Iowa State Agr. Soc. (1882), p. 329.
1890. *Lynx rufus* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 41.
1910. *Lynx rufus* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 218.

The Wildcat was formerly found throughout Iowa, and it is barely possible that stragglers may still appear in the bluffs along the Mississippi River in the northeastern part of the state.

Osborn (1905) considered the species near extinction. For Nebraska, Swenk (1915, p. 853) included it as "Rare eastwardly." Stephens (1922) writes that specimens, taken here and there throughout northwestern Iowa, are probably this animal. In Minnesota "It is more common than is generally known along the Mississippi River bluffs where it finds congenial habitat among the cliffs" (Surber, 1932, p. 56).

A specimen, bearing the locality record "Iowa" and without the date of capture is contained in the Iowa State College Museum.

Specimens examined:

"Iowa"; museum No. 1; Iowa State College Museum.

Order 5. Rodentia

Family Sciuridae

SOUTHERN WOODCHUCK

Marmota monax monax (Linnaeus)

1758. [*Mus*] *monax* Linnaeus, Syst. Nat., ed. 10, vol. 1, p. 60.
    Type Locality: Maryland.
1871. *Arctomys monax* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 190.

1883. *Arctomys monax* Goding, Iowa State Agr. Soc. (1882), p. 331.
1890. *Arctomys monax* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.
1910. *Arctomys monax* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 214.
1918. *Marmota monax monax* Stoner, Iowa Geol. Survey, Bul. No. 5, p. 45.
1921. *Marmota monax monax* Gabrielson, Proc. Iowa Acad. Sci., vol. 28, p. 148.

The Southern Woodchuck, formerly south of Iowa, is now found throughout the state. The northward extension of its range was most rapid along wooded streams; hence, the earliest observations of this mammal were made along the larger streams of the Missouri and Mississippi River systems. Perhaps this is what led Stoner (1918, p. 45), being most familiar with the Mississippi Valley, to write: "The woodchuck is a common rodent throughout the eastern half of the state, but is seldom met with in the extreme western counties."

Spurrell (1917) was informed that the woodchuck was present in Sac County when the settlers first came in 1854. While Allen (1871) further south but higher in the Missouri-Mississippi watershed did not record the species as late as 1867. A reported observation of the animal in Davis County was accepted by Allen during the same investigation. It was not until the 1900's that the woodchuck appeared at Atlantic, Cass County (Pellett, letter, 1936). Spurrell (1917) saw an increased number of these animals in Sac County after 1905. It was not observed by Ruthven and Wood (1912) in Clay and Palo Alto counties during the expedition of 1907. In about 1916 the first woodchucks appeared in the lake region of Dickinson County (Stephens, 1922). Unfortunately, the specimen from Ames, Story County, in the Iowa State College, bears no date of capture.

Published records of museum specimens: Sac Co., Wall Lake, A. H. Howell (1915), 1, University of Iowa Museum; Johnson Co., A. H. Howell (1915), 8, University of Iowa Museum.

Specimens examined:

Story County, Ames, museum No. 34; Iowa State College Museum.

THIRTEEN-STRIPED GROUND SQUIRREL
Citellus tridecemlineatus tridecemlineatus (Mitchill)

1821. *Sciurus tridecem-lineatus* Mitchill, Med. Repos., N. S. vol. 6 (21), p. 248. Type Locality: Central Minnesota.
1871. *Spermophilus tridecem-lineatus* Allen, Proc. Boston Soc. Nat. Hist., Vol. 13, p. 189.
1883. *Spermophilus tridecem-lineatus* Goding, Iowa State Agr. Soc. (1882), p. 331.
1890. *Spermophilus tridecemlineatus* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.
1910. *Spermophilus tridecemlineatus* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 214.
1912. *Citellus tridecemlineatus* Ruthven and Wood, Proc. Iowa Acad. Sci., vol. 19, p. 203.
1918. *Citellus tridecemlineatus tridecemlineatus* Stoner, Iowa Geol. Survey, Bul. No. 5, p. 29.
1921. *Citellus tridecemlineatus tridecemlineatus* Gabrielson, Proc. Iowa Acad. Sci., vol. 28, p. 147.

The Thirteen-striped Ground Squirrel is a common resident throughout the open grasslands of the state. The species is most abundant in the northern part of the state.

A specimen of this species from Luxemburg, Dubuque County, is contained in the Field Museum of Natural History (Simms, letter, 1936). This is more than likely the specimen to which Cory (1912, p. 144) refers. Surber has a specimen from Fairport, Muscatine County, in his private collection (letter, 1936). George G. Goodwin, Assistant Curator, writes of 17 specimens from Webb, Clay County, in the American Museum of Natural History (letter, 1936).

Published records of museum specimens: Clay and Palo Alto counties, Ruthven and Wood (1912), 27, Museum of Zoology, University of Michigan.

Specimens examined:

Story County, Ames; 3; Iowa State College Museum.

FRANKLIN GROUND SQUIRREL
Citellus franklini (Sabine)

1822. *Arctomys franklini* Sabine, Trans. Linn Soc., vol. 13, p. 587.
Type Locality: Vicinity of Carlton House, Saskatchewan, Canada.
1871. *Spermophilus franklini* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 189.
1883. *Spermophilus franklini* Goding, Iowa State Agr. Soc. (1882), p. 331.
1890. *Spermophilus franklini* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.
1910. *Spermophilus franklini* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 214.
1912. *Citellus franklini* Ruthven and Wood, Proc. Iowa Acad. Sci,. vol. 19, p. 204.
1918. *Arctomys franklinii* (sic) Stoner, Iowa Geol. Survey, Bul. No. 5, p. 36.
1921. *Citellus franklini* Gabrielson, Proc. Iowa Acad. Sci., vol. 28, p. 147.

The Franklin Ground Squirrel is found throughout the state, originally inhabiting the open prairie and later spreading over the areas opened by agriculture. The irregular occurrence of this species in a given locality may be due to migration. It is not nearly so numerous as *tridecimlineatus*.

Surber (letter, 1936), Superintendent of Fish Propagation, Minnesota Department of Conservation, writes that a specimen of this species from Fairport, Muscatine County, is contained in his private collection. There are four specimens from Webb, Clay County, and one from Iowa City, Johnson County, in the Amreican Museum of Natural History (Goodwin, letter, 1936).

Published records of museum specimens: Clay Co., Ruthven and Wood (1912), 10, Museum of Zoology, University of Michigan.

GRAY EASTERN CHIPMUNK
Tamias striatus griseus Mearns

1891. *Tamias striatus griseus* Mearns, Bul. Amer. Mus. Nat. Hist., vol. 3, p. 231.
Type Locality: Fort Snelling, Hennepin County, Minnesota.
1871. *Tamias striatus* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 189.
1883. *Tamias striatus* Goding, Iowa State Agr. Soc. (1882), p. 331.
1890. *Tamias striatus* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.
1910. *Tamias striatus griseus* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 214.
1918. *Tamias striatus griseus* Stoner, Iowa Geol. Survey, Bul. No. 5, p. 27.
1921. *Tamias striatus griseus* Gabrielson, Proc. Iowa Acad. Sci., vol. 28, p. 147.

The Gray Eastern Chipmunk is rather generally distributed throughout the wooded parts of the state. Early reports indicate that it is not nearly so numerous as it was at the time of settlement. At present it appears to be locally common in eastern and southeastern Iowa and decreasing in numbers wherever the wooded cover is being removed.

A specimen from Cedar Rapids, Linn County, in the Coe College Museum was examined by Cory (1912) and referred to *griseus*. There are no specimens of *Tamias* in the Coe College Museum at the present time (Stiles, letter, 1937).

Published records of museum skins: Story Co., Ames, A. H. Howell (1929), 1, collection of the United States Biological Survey; Des Moines Co., Burlington, A. H. Howell (1929), 29, collection of the United States Biological Survey; Linn Co., Cedar Rapids, A. H. Howell (1929), 1, Field Museum of Natural History; Floyd Co., Charles City, A. H. Howell (1929), 1, University of Iowa Museum; Henry Co., Hillsboro and Wayland, A. H. Howell (1929), 2, University of Iowa Museum.

SOUTHERN RED SQUIRREL
Sciurus hudsonicus loquax Bangs

1896. *Sciurus hudsonicus loquax* Bangs, Proc. Biol. Soc. Washington, vol. 10, p. 161. Type Locality: Liberty Hill, New London County, Connecticut.

1871. *Sciurus hudsonius* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 188.

1883. *Sciurus hudsonius* Goding, Iowa State Agr. Soc. (1882), p. 331.

1910. *Sciurus hudsonicus loquax* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 214.

1918. *Sciurus hudsonicus loquax* Stoner, Iowa Geo. Survey, Bul. No. 5, p. 25.

The exact range of the Southern Red Squirrel in Iowa is undetermined, but is probably over the southern half of the state. It is locally common, especially toward the southeast.

Cory (1912) refers a specimen taken at Knoxville, Marion County, to this subspecies. No doubt this is the Knoxville specimen now reposing in the Field Museum of Natural History (Simms, letter, 1936). The records of Stoner (1918) and others are questionable since no attempt is made to distinguish between *loquax* and *minnesota*.

MINNESOTA RED SQUIRREL
Sciurus hudsonicus minnesota Allen

1899. *Sciurus hudsonicus minnesota* Allen, Amer. Nat., vol. 33, p. 640. Type Locality: Fort Snelling, Hennepin County, Minnesota.

The range of the Minnesota Red Squirrel is undetermined in Iowa, but is probably over the northern half of the state. It is of local abundance, increasing in numbers toward the extreme northeast.

The only authentic record for *minnesota* in Iowa is based on a specimen taken at Charles City, Floyd County (Stoner, 1917). Identification of this specimen was made by A. H. Howell of the United States Biological Survey. Stephens' (1922) record of *loquax* for Dickinson County is probably a misidentification of *minnesota*. The records of *loquax* for Dickinson and Cerro Gordo counties by Stoner (1918) are questionable; Stoner's *loquax* for Charles City, Floyd County, was correctly referred to *minnesota* by Howell as has already been mentioned.

NORTHERN GRAY SQUIRREL
Sciurus carolinensis leucotis (Gapper)

1830. *Sciurus leucotis* Gapper, Zool. Journ., vol. 5, p. 206.
Type Locality: Between York and Lake Simcoe, Ontario, Canada.
1871. *Sciurus carolinensis* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 188.
1883. *Sciurus carolinensis* Goding, Iowa State Agr. Soc. (1882), p. 331.
1890. *Sciurus carolinensis* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.
1910. *Sciurus carolinensis* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 214.
1918. *Sciurus carolinensis leucotis* Stoner, Iowa Geol. Survey, Bul. No. 5, p. 22.

The geographic distribution of the Northern Gray Squirrel in Iowa is not well known, but is probably confined to the eastern part of the state, especially to the north. The occurrence of this squirrel is irregular in a given locality due to mass migrations. Many gray squirrels were seen about Clinton, Clinton County, in the '60's, but none were present by 1915 (Spurrell, 1915). The Northern Gray Squirrel is reported as abundant in northeastern Iowa by Stoner (1918), but such large populations are not present today. Surber (1932, p. 61) writes for Minnesota: "This squirrel is common in the oak and hard maple forests along the eastern border of the state from the Iowa line north into Pine County . . ."

WESTERN FOX SQUIRREL
Sciurus niger rufiventer (Geoffroy)

1803. *Sciurus rufiventer* Geoffroy, Catl. Mamm. Mus. Nat. Hist., Paris, p. 176.
Type Locality: Mississippi Valley, exact locality unknown.
1871. *Sciurus ludovicianus* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 188.
1883. *Sciurus vulpinus* Goding, Iowa State Agr. Soc. (1882), p. 331.
1890. *Sciurus cinereus* var. *ludovicianus* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.
1910. *Sciurus ludovicianus* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 214.
1918. *Sciurus niger rufiventer* Stoner, Iowa Geol. Survey, Bul. No. 5, p. 19.
1921. *Sciurus niger rufiventer* Gabrielson, Proc. Iowa Acad. Sci., vol. 28, p. 147.

The Western Fox Squirrel is common throughout the wooded parts of the state, even spreading into isolated farm groves on the prairie.

Cory (1912) refers five specimens from Knoxville, Marion County, to this subspecies. The Knoxville specimens examined by Cory are contained in the Field Museum of Natural History (Simms, letter, 1936). Mr. George G. Goodwin, Assistant Curator, writes of two specimens from Webb, Clay County, referable to *rufiventer*, in the American Museum of Natural History (letter, 1936). A specimen, No. 23a, taken at Pacific Junction, Mills County, and another, No. 19a, taken at Duncan, Hancock County, have been identified as this subspecies by A. H. Howell.

Specimens examined:

Story County, Ames; museum No. 1; Iowa State College Museum.
Mills County, Pacific Junction; No. 23a; collection of the United States Biological Survey.
Hancock County, Duncan; No. 19a; collection of the United States Biological Survey.

SMALL EASTERN FLYING SQUIRREL
Glaucomys volans volans (Linnaeus)

1758. [*Mus*] *volans* Linnaeus, Syst. Nat., ed. 10, vol. 1, p. 63.
Type Locality: Virginia.
1871. *Pteromys volucella* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 189.
1883. *Pteromys volucellus* (sic) Goding, Iowa State Agr. Soc. (1882), p. 331.
1890. *Sciuropterus volans* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.
1910. *Sciuropterus volans* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 215.
1918. *Gaucomys volans volans* Stoner, Iowa Geol. Survey, Bul. No. 5, p. 17.
1921. *Glaucomys volans volans* Gabrielson, Proc. Iowa Acad. Sci., vol. 28, p. 147.

The Small Eastern Flying Squirrel is found locally throughout the wooded parts of the state, excepting the northwestern corner, where it appears to be rare or absent.

There is a specimen of this squirrel from Iowa City, Johnson County, in the American Museum of Natural History (Goodwin, letter, 1936); and another from Knoxville, Marion County, in the Field Museum of Natural History (Simms, letter, 1936).

Published records of museum specimens: Henry Co., Hillsboro, A. H. Howell (1918), 2, collection of the United States Biological Survey; Johnson County, Iowa City, A. H. Howell (1918), 1, private collection of Dayton Stoner; Union Co., Thayer, A. H. Howell (1918), 2, private collection of Dayton Stoner; Marion Co., Knoxville, A. H. Howell (1918), 3, collection of the Biological Survey.

Specimens examined:

Story County, Ames; 1; Iowa State College collection.

Family Geomyidae

SHAW POCKET GOPHER
Geomys bursarius (Shaw)

1800. *Mus bursarius* Shaw, Trans. Linn. Soc., vol. 5, p. 227.
Type Locality: Not known, somewhere in the upper Mississippi Valley.
1871. *Geomys bursarius* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 190.
1883. *Geomys bursarius* Goding, Iowa State Agr. Soc. (1882), p. 331.
1890. *Geomys bursarius* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.
1910. *Geomys bursarius* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 212.
1912. *Geomys bursarius* Ruthven and Wood, Proc. Iowa Acad. Sci., vol. 19, p. 204.
1918. *Geomys bursarius bursarius* (sic) Stoner, Iowa Geol. Survey, Bul. No. 5, p. 108.
1921. *Geomys bursarius bursarius* (sic) Gabrielson, Proc. Iowa Acad Sci., vol. 28, p. 148.

Numerous "gopher mounds" throughout the grasslands of the state indicate the wide distribution of the Shaw Pocket Gopher. This mammal was not so numerous at the time of settlement as it is today. Early settlers informed Spurrell (1917) that the gopher was not so common during the early days in Sac County. Cultivation and general opening up of the land has provided for increased numbers by extension of the natural range.

Specimens examined:

Story County, Ames; museum No. 1; Iowa State College Museum.

Family HETEROMYIDEA

DUSKY POCKET MOUSE
Perognathus flavescens perniger Osgood

1904. *Perognathus flavescens perniger* Osgood, Proc. Biol. Soc. Washington, vol. 17, p. 127.
Type Locality: Vermilion, Clay County, South Dakota.

This represents the first record of the Dusky Pocket Mouse, *Perognathus flavescens perniger,* for Iowa. Locality records are not sufficient to permit determination of the range, but it will probably prove to be over the western two-thirds of the state. Two specimens of *P. f. perniger* are in the Iowa State College collection, one from Guthrie Center, Guthrie County, and the other from Oakland, Pottawattamie County. These specimens were referred to *perniger* by A. H. Howell.

Specimens examined:
Guthrie County, Guthrie Center; 1; Iowa State College collection.
Pottawattamie County, Oakland; 1; Iowa State College collection.

Family CASTORIDAE

MISSOURI RIVER BEAVER
Castor canadensis missouriensis Bailey

1919. *Castor canadensis missouriensis* Bailey, Journ. Mamm., vol. 1, p. 32.
Type Locality: Apple Creek, 7 miles east of Bismarck, Burleigh County, North Dakota.

The Missouri River Beaver is found irregularly over the Missouri River watershed, especially in the northwestern part of the state. Food and drainage problems frequently check the permanency of its establishment in given regions; nevertheless, its numbers are stable to increasing.

References to *canadensis* typicus for Iowa cannot be presented as misidentification of *missouriensis,* for it is possible that the beaver inhabiting extreme northeastern Iowa today and formerly over the eastern part of the state may be of the type form.

History indicates that the beaver became scarce or extinct in the state during the '90's. Osborn (1890) considered them rare; Van Hyning and Pellett (1910, p. 214) believed them to have "—become extinct some time in the nineties." There is no doubt but that the heaver has reestablished itself in respect to numbers, but its once complete extinction is subject to question.

Two specimens taken in December, 1936, at Lakeview, Sac County, were referred to this subspecies by A. H. Howell. An unidentified specimen of beaver, taken on February 12, 1887, in Cherokee County, is contained in the Milwaukee Public Museum (Gromme, letter, 1936).

Specimens examined:
Sac County, Lakeview; Nos. 43a and 25a (skulls); Iowa State College collection.

Family Cricetidae

SHORT-EARED GRASSHOPPER MOUSE
Onychomys leucogaster breviauritus Hollister

1913. *Onychomys leucogaster breviauritus* Hollister, Proc. Biol. Soc. Washington, vol. 26, p. 216.
Type Locality: Fort Reno, Canadian County, Oklahoma.

The only record of the Short-eared Grasshopper Mouse for Iowa is based on a specimen taken in Dickinson County and contained in the Museum of Zoology at the University of Michigan (Dice, 1924, p. 66). The occurrence of this mouse may prove to be accidental with continued collection. Dice's record is considerably off the known range, since Hollister (1914, p. 453) writes: "Eastern Nebraska, eastern and south-central Kansas, and middle Oklahoma. From Neligh, Nebraska, and Fort Riley and Neosha Falls, Kansas, west and south to Kinsley, Kansas, and to Woodward and Fort Reno, Oklahoma. Entirely within the Carolinean and Austroriparian faunas of the Austral region." Swenk (1915, p. 852) considers it "Uncommon eastwardly" in Nebraska.

PRAIRIE HARVEST MOUSE
Reithrodontomys megalotis dychei (Allen)

1895. *Reithrodontomys dychei* Allen, Bull. Amer. Mus. Nat. Hist., vol. 7, p. 120.
Type Locality: Lawrence, Douglas County, Kansas.
1890. *Ochetodon humilis* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.
1912. *Reithrodontomys griseus* Ruthven and Wood, Proc. Iowa Acad. Sci., vol. 19, p. 204 (Not Bailey).
1918. *Reithrodontomys megalotis dychei* Stoner, Iowa Geol. Survey, Bul. No. 5, p. 76.
1922. *Reithrodontomys megalotis dychei* Stephens, Okoboji Protective Assoc., Bul. No. 18, p. 55.

The Prairie Harvest Mouse is found throughout the grasslands of the state.

A map by A. H. Howell (1914) presents the known range of this mouse in Iowa as everywhere southwest of a line from the northwest corner of the state southeast to the vicinity of Waterloo and south to Keokuk. However, there is no doubt but that this species is found in suitable locations over the remainder of the state. Surber (1932) reports two specimens from Homer, Winona County, on the Mississippi River in Minnesota. There are also four specimens from further south on the Mississippi River at Fairport, Muscatine County, Iowa, in the private collection of Thaddeus Surber, Superintendent of Fish Propagation, Minnesota Department of Conservation (letter, 1936). Pellett (1912) finds this mouse common near Atlantic, Cass County.

Five specimens referable to *dychei* from Atlantic, Cass County, are contained in the Milwaukee Public Museum (Gromme, letter, 1936). Stoner (1918) claims specimens from Iowa City, Wall Lake, Atlantic, Ottumwa, Tama, Logan and Jefferson but fails to give their location. Two specimens from Ames, Story County, were referred to *dychei* by A. H. Howell.

Published records of museum specimens: Cass Co., Atlantic, A. H. Howell (1914), 2, collection of the United States Biological Survey;

Henry Co., Hillsboro, A. H. Howell (1914), 2, collection of the United States Biological Survey; Palo Alto Co., A. H. Howell (1914), 1, Museum of Zoology at the University of Michigan.

Specimens examined:

Story County, Ames; 2; Iowa State College collection.

BAIRD WHITE-FOOTED MOUSE
Peromyscus maniculatus bairdi (Hoy and Kennicott)

1857. *Mus bairdii* Hoy and Kennicott, in Kennicott, Agricultural Report, United States Patent Patent Office, 1856, p. 92.
Type Locality: Bloomington, McClean County, Illinois.

1871. *Hesperomys michiganensis* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 193.

1883. *Hesperomys michiganensis* Goding, Iowa State Agr. Soc. (1882), p. 330.

1890. *Hesperomys michiganensis* Osborn, Proc. Iowa Acad. Sci., vol 1, p. 43.

1910. *Peromyscus maniculatus* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 213.

1912. *Peromyscus maniculatus bairdii* Ruthven and Wood, Proc. Iowa Acad. Sci., vol. 19, p. 204.

1918. *Peromyscus maniculatus bairdi* Stoner, Iowa Geol. Survey, Bul. No. 5, p. 73.

The Baird White-footed Mouse is common throughout the state.

Stoner (1918) claims specimens from Corning, Jefferson, Thayer, Wall Lake, Ottumwa, Melvin, Waukon, Rodman, Ocheyedan, Logan and Homestead, but fails to give their location.

Four specimens from Fairport, Muscatine County, are contained in the private collection of Thaddeus Surber, Superintendent of Fish Propagation, Minnesota Department of Conservation (letter, 1936). George G. Goodwin, Assistant Curator, writes of 20 specimens from Iowa City, Johnson County, in the American Museum of Natural History (letter, 1936). There is a single specimen from Knoxville, Marion County, in the Field Museum of Natural History (Simms, letter, 1936).

Published records of museum specimens: Clay Co., Osgood (1909), 5, Museum of Zoology at the University of Michigan; Palo Alto Co., Osgood (1909), 4, Museum of Zoology at the University of Michigan; Marion Co., Knoxville, Osgood (1909), 14, collection of the United States Biological Survey.

Specimens examined:

Boone County, Jordan; No. 33a; Iowa State College collection.
Decatur County, High Point; 2; Iowa State College collection.
Lee County, Fort Madison; No. 20a; Iowa State College collection.
Muscatine County, Fairport; 1; Museum of Natural History, University of Minnesota.

(All specimens of *bairdi* in the Iowa State College collection were identified by A. H. Howell of the United States Biological Survey.)

NORTHERN WHITE-FOOTED MOUSE
Peromyscus leucopus noveboracensis (Fischer)

1829. *Mus [sylvaticus] δ noveboracensis* Fischer, Synopsis Mamalium, p. 318.
Type Locality: New York.

1871. *Hesperomys leucopus* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 192.

1883. *Hesperomys leucopsus* (sic) Goding, Iowa State Agr. Soc. (1882), p. 330.

1890. *Hesperomys leucopus* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.

1910. *Peromyscus leucopus noveboracensis* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 213.

1918. *Peromyscus leucopus noveboracensis* Stoner, Iowa Geol. Survey, Bul. No. 5, p. 69.

1921. *Peromyscus leucopus noveboracensis* Gabrielson, Proc. Iowa Acad. Sci., vol. 28, p. 148.

The Northern White-footed Mouse is common throughout all Iowa.

Stoner (1918) claims specimens from Wayland, Hayfield, Burlington, Fairport, Thayer, Wall Lake, Iowa City, Charles City, Homestead and Newton, but fails to give their location.

There are seven specimens of *noveboracensis* from Fairport, Muscatine County, in the private collection of Thaddeus Surber, Superintendent of Fish Propagation, Minnesota Department of Conservation (letter, 1936). The Field Museum of Natural History contains one specimen from Knoxville, Marion County, and three from Iowa City, Johnson County (Simms, letter, 1936).

Published records of museum specimens: Des Moines Co., Burlington, Osgood (1909), 57, collection of the United States Biological Survey; Pottawattamie Co., Council Bluffs, Osgood (1909), 5, collection of the United States Biological Survey; Marion Co., Knoxville, Osgood (1909), 10, collection of the United States Biological Survey; Dallas Co., Osgood (1909), 1, collection of the United States Biological Survey.

Specimens examined:

Decatur County, High Point; 3; Woodland 3; Iowa State College collection.

Hamilton County, Jewell; Nos. 16a, 15a, 8a, 4a, 5a, and 1a; Iowa State College collection.

Story County, Ames; No. 35a, No. 32a, plus 4; Iowa State College collection.

(All specimens of *noveboracensis* in the Iowa State College collection were identified by A. H. Howell.)

GOSS LEMMING MOUSE
Synaptomys cooperi gossii (Coues)

1877. *Arvicola (Synaptomys) gossii* Coues, Monogr. N. Amer. Rodentia, p. 235 (published as a synonym of *Synaptomys cooperi*, but name stated to apply to Kansas specimens, description p. 236).

Type Locality: Neosho Falls, Woodson County, Kansas.

1890. *Synaptomys cooperi* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.

1918. *Synaptomys cooperi gossii* Stoner, Iowa Geol. Survey, Bul. No. 5, p. 106.

The range of the Goss Lemming Mouse in Iowa has not been completely determined, but it appears to be confined to the wet meadows and marsh edges of the southern half of the state. In respect to the scarcity of material, A. B. Howell (1927, p. 3) writes: "Until more specimens are obtained, further progress in the proper understanding of the relationship of several races can hardly be expected."

Specimens are recorded from Logan, Harrison County, and from Fairport, Muscatine County, by Stoner (1918). The specimen from Fairport is in the private collection of Thaddeus Surber, Superintendent of Fish Propagation, Minnesota Department of Conservation (letter,

1936). There is a record of this mouse for Dickinson County by Stephens (1922, p. 56).

Published records of museum specimens: Henry Co., Hillsboro, A. B. Howell (1927), 1, collection of the United States Biological Survey; Linn Co., Marion, A. B. Howell (1927), 1, Museum of Comparative Zoology; Marion Co., Knoxville, A. B. Howell, 1, collection of the Biological Survey.

PENNSYLVANIA MEADOW MOUSE
Microtus pennsylvanicus pennsylvanicus (Ord)

1815. *Mus pennsylvanica* Ord, Guthrie's Geography, 2d Amer. ed., vol. 2, p. 292. Type Locality: Meadows below Philadelphia, Pennsylvania.

1871. *Arvicola riparius* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 193.

1883. *Arvicola riparia* (sic) Goding, Iowa State Agr. Soc. (1882), p. 330.

1890. *Arvicola riparius* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.

1910. *Microtus pennsylvanicus* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 213.

1918. *Microtus pennsylvanicus pennsylvanicus* Stoner, Iowa Geol. Survey, Bul. No. 5, p. 78.

1921. *Microtus pennsylvanicus pennsylvanicus* Gabrielson, Proc. Iowa Acad. Sci., vol. 28, p. 148.

The Pennsylvania Meadow Mouse is found in all parts of Iowa, especially in wet meadows and marsh borders.

Stoner (1918) claims specimens from Melvin, Thayer, Atlantic, Jefferson, Waukon, Charles City and Iowa City, but fails to give their location. Authentic records are given for Marshall County by Gabrielson (1921) and for Sac County by Spurrell (1917).

A single specimen from Fairport, Muscatine County, is contained in the private collection of Thaddeus Surber, Superintendent of Fish Propagation, Minnesota Department of Conservation (letter, 1936). George G. Goodwin, Assistant Curator, writes of three specimens from Webb, Clay County, in the American Museum of Natural History (letter, 1936). There are three specimens from Luxemburg, Dubuque County, and two from Knoxville, Marion County, in the Feild Museum of Natural History (Simms, letter, 1936). Specimens from Clay County are contained in the Museum of Zoology, University of Michigan (Burt, letter, 1937).

Published records of museum specimens: Marion Co., Knoxville, Bailey (1900), 2, Field Museum of Natural History.

Specimens examined:

Dickinson County, Arnold's Park; No. 11a; Okoboji; No. 10a; Iowa State College collection.

Palo Alto County, Ruthven; No. 9a; Iowa State College collection.

Story County, Ames; No. 6a, plus 1; Iowa State College collection.

Muscatine County, Fairport; 7; Museum of Natural History, University of Minnesota.

(All specimens in the Iowa State College collection were referred to *M. p. pennsylvanicus* by A. H. Howell.)

PRAIRIE MEADOW MOUSE
Microtus ochrogaster (Wagner)

1842. *Hypudaeus ochrogaster* Wagner, Schreber's Saughthiere, Suppl., vol. 3, p. 592. Type locality: America.

1871. *Arvicola austera* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 194.
1883. *Arvicola austera* Goding, Iowa State Agr. Soc. (1882), p. 330.
1890. *Arvicola austerus* (sic) Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.
1910. *Microtus austerus* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 213.
1918. *Microtus ochrogaster ochrogaster* (sic) Stoner, Iowa Geol. Survey, Bul. No. 5, p. 87.
1921. *Microtus ochrogaster ochrogaster* (sic) Gabrielson, Proc. Iowa Acad. Sci., vol. 28, p. 148.

The Prairie Meadow Mouse is found throughout the state, exhibiting a preference for a drier habitat than that chosen by *pennsylvanicus*.

Stoner (1918) claims specimens from Newton, Ottumwa, Monticello, Wall Lake, Waukon, Logan, Corning, Melvin, Atlantic and Homestead, but fails to give their location. There are 20 specimens from Iowa City, Johnson County, in the American Museum of Natural History (Goodwin, letter, 1936). The Field Museum of Natural History contains three specimens from Knoxville, Marion County (Simms, letter, 1936). Gabrielson (1921) gives an authentic record for Marshall County.

Published records of museum specimens: Jefferson Co., Fairfield, Bailey (1900), 1, collection of the United States Biological Survey; Marion Co., Knoxville, 93, collection of the United States Biological Survey.

Specimens examined:

Story County, Ames; Nos. 26a, 27a, 28a, 30a, 31a, plus 5; Iowa State College collection.

Decatur County, Woodland; 3; Iowa State College collection.

(All specimens in the Iowa State College collection were identified by A. H. Howell.)

WOODLAND PINE MOUSE
Pitymys nemoralis (Bailey)

1898. *Microtus pinetorum nemoralis* Bailey, Proc. Biol. Soc. Washington, vol. 12, p. 89.
Type Locality: Stilwell, Adair County, Oklahoma.
1871. *Arvicola pinetorum* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 194.
1910. *Microtus nemoralis* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 213.
1918. *Microtus pinetorum nemoralis* Stoner, Iowa Geol. Survey, Bul. No. 5, p. 91.

The Woodland Pine Mouse is found in the southern one-half of Iowa, exhibiting a preference for brushy fence rows and woodland borders.

Stoner (1918) claims specimens from Thayer, Ottumwa and Iowa City, but fails to give their location. A single specimen of *nemoralis* is contained in the private collection of Thaddeus Surber, Superintendent of Fish Propagation, Minnesota Department of Conservation (letter, 1936).

Published records of museum specimens: Pottawattamie Co., Council Bluffs, Bailey (1900), 1, collection of the United States Biological Survey.

COMMON MUSKRAT
Ondatra zibethica zibethica (Linnaeus)

1766. [*Castor*] *zibethicus* Linnaeus, Syst. Nat., ed. 12, vol. 1, p. 79.
Type Locality: Eastern Canada.
1871. *Fiber zibethicus* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 194.
1883. *Fiber zibethicus* Goding, Iowa State Agr. Soc. (1882), p. 331.

1890. *Fiber zibethicus* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.
1910. *Fiber zibethicus* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 213.
1912. *Fiber zibethicus* Ruthven and Wood, Proc. Iowa Acad. Sci., vol. 19. p. 204.
1918. *Fiber zibethicus zibethicus* Stoner, Iowa Geol. Survey, Bul. 5, p. 96.
1921. *Fiber zibethicus zibethicus* Gabrielson, Proc. Iowa Acad. Sci., vol. 28, p. 148.

The determined range of the Common Muskrat for Iowa is everywhere east of a line from Minnesota south through Hayfield, Hancock County, and continuing south through Ames, Story County, southeast through Ottumwa, Wapello County, to Missouri. Undoubtedly this western limit of distribution will be extended with continued collection, but the available material does not warrant such alteration at present.

The record given by Cory (1912) for Mayfield is a misspelling of Hayfield, Hancock County. These six specimens are in the Field Museum of Natural History (Simms, letter, 1936). Gabrielson (1921) offers an authentic record for Marshall County. The *Fiber zibethicus* = *Ondatra z. zibethica* of Ruthven and Wood (1912) for Clay and Palo Alto counties is doubtful, and the records of Spurrell (1917) for Sac County and of Stephens (1922) for Dickinson County are also subject to question for all of these records fall within what appears to be *cinnamomina* range (Hollister, 1911). Stoner (1918) offers no definite records.

Published records of museum specimens: Des Moines Co., Burlington, Hollister (1911), 1, collection of the United States Biological Survey.

Specimens examined:

Story County, Ames; No. 37a; collection of the United States Biological Survey.

Hamilton County, Jewell; 6; collection of the United States Biological Survey.

(All of the above specimens were referred to *O. z. zibethica* by A. H. Howell.)

GREAT PLAINS MUSKRAT
Ondatra zibethica cinnamomina (Hollister)

1910. *Fiber zibethicus cinnamominus* Hollister, Proc. Biol. Soc. Washington, vol. 23, p. 125.
Type Locality: Wakeeney, Tergo County, Kansas.
1918. *Fiber zibethicus cinnamominus* Stoner, Iowa Geol. Survey, Bul. 5, p. 105.

The Great Plains Muskrat is found in western Iowa, but the exact limits of distribution have not been determined. Stoner (1918) includes *cinnamomina* on the basis of Hollister's (1911) record for Knoxville, Marion County. Stoner (1918, p. 106) writes: "The writer has not obtained specimens of this muskrat, but collectors and trappers should be on the lookout for it in western Iowa, where the area of intergradation occurs."

Published records of museum specimens: Marion Co., Knoxville, Hollister (1911), 1, collection of the United States Biological Survey.

HOUSE MOUSE
Mus musculus musculus Linnaeus

1758. [*Mus*] *musculus* Linnaeus, Syst. Nat., ed. 10, vol. 1, p. 62.
Type Locality: Upsala, Sweden.

1883. *Mus musculus* Goding, Iowa State Agr. Soc. (1882), p. 330.
1890. *Mus musculus* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.
1910. *Mus musculus* Van Hyning and Pellett, Proc. Icwa Acad. Sci., vol. 17, p. 214.
1912. *Mus musculus* Ruthven and Wood, Proc. Iowa Acad. Sci., vol. 19, p. 204.
1918. *Mus musculus* Stoner, Iowa Geol. Survey, Bul. No. 5, p. 52.
1921. *Mus musculus* Gabrielson, Proc. Iowa Acad. Sci., vol. 28, p. 148.

The House Mouse is common about buildings, showing a tendency to spread to the fields over the state.

NORWAY RAT
Rattus norvegicus (Erxleben)

1777. [*Mus*] *norvegicus* Erxleben, Syst. Regni. Anim., vol. 1, p. 381.
Type Locality: Norway.
1883. *Mus decumanus* Goding, Iowa State Agr. Soc. (1882), p. 331.
1890. *Mus decumanus* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.
1910. *Mus decumanus* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 214.
1918. *Rattus norvegicus* Stoner, Iowa Geol. Survey, Bul. No. 5, p. 54.
1921. *Rattus norvegicus* Gabrielson, Proc. Iowa Acad. Sci., vol. 28, p. 148.

The Norway Rat is common about buildings in all parts of the state.

This rat was introduced to eastern United States in 1775, and upon establishment spread to all parts of the country in shipments. Early settlers informed Spurrell (1917) that the first barn rat same to Sac County in a box of goods from New York in 1858. This rat was killed, and the species did not appear again until about 1868.

Family Zapodidae

PRAIRIE JUMPING MOUSE
Zapus hudsonius campestris Preble

1899. *Zapus hudsonius campestris* Preble, North Amer. Fauna, No. 15, p. 20.
Type Locality: Bear Lodge Mountains, Crook County, Wyoming.
1871. *Jaculus hudsonius* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 192.
1883. *Jaculus hudsonius* Goding, Iowa State Agr. Soc. (1882), p. 330.
1890. *Zapus hudsonius* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.
1910. *Zapus hudsonius campestris* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 212.
1918. *Zapus hudsonius campestris* Stoner, Iowa Geol. Survey, Bul. No. 5, p. 124.

The Prairie Jumping Mouse is irregularly distributed throughout the state, showing a tendency towards local abundance.

The specimens contained in the Iowa State College collection represent the first authentic records of *campestris* for the state. They were referred to this subspecies by A. H. Howell.

Specimens examined:

Palo Alto County, Ruthven; 1; Iowa State College collection.
Story County, Ames; 1; Iowa State College collection.
Ida County, Arthur; 1; Iowa State College collection.

Order 6. LAGOMORPHA

Family LEPORIDAE

WHITE-TAILED JACK RABBIT
Lepus townsendii campanius Hollister

1837. *Lepus campestris* Bachman, Journ. Acad. Nat. Sci., Philadelphia, vol. 7, p. 349. Type Locality: Plains of the Saskatchewan, Canada.
1890. *Lepus campestris* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.
1910. *Lepus campestris* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 212.
1912. *Lepus campestris* Ruthven and Wood, Proc. Iowa Acad. Sci., vol. 19, p. 204.
1918. *Lepus townsendii campanius* Stoner, Iowa Geol. Survey, Bul. No. 5, p. 131.
1921. *Lepus townsendi* (sic) *campanius* Gabrielson, Proc. Iowa Acad. Sci., vol. 28, p. 148.

The White-tailed Jack Rabbit is found throughout the state, being most numerous in the northern counties.

This species has extended its range over Iowa from the northwest. Early settlers in Sac County informed Spurrell (1917) that the jack rabbit was not present upon their arrival in 1854 and did not appear until 1868. It was considered occasional in Dickinson County by Mosher (1882). Evidently it had not been observed very frequently by the summer of 1867 since it does not appear in Allen's (1871) list. The species increased noticeably in the northern counties during the '90's (Osborn, 1892, and Nutting, 1895). Its appearance in Clinton County is noted for 1905 (Spurrell, 1917). The extension to the northeastern part of the state was a little slower since it did not appear there until about 1911 (Stoner, 1917).

Specimens examined:

Story County, Ames; Museum No. 9 and No. 49; Iowa State College Museum.

MEARNS COTTONTAIL
Sylvilagus floridanus mearnsi (Allen)

1894. *Lepus sylvaticus mearnsii* Allen, Bull. Amer. Mus. Nat. Hist., vol 6, p. 171. Type Locality: Fort Snelling, Hennepin County, Minnesota.
1871. *Lepus sylvaticus* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 194.
1883. *Lepus sylvaticus* Goding, Iowa State Agr. Soc. (1882), p. 331.
1890. *Lepus sylvaticus* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.
1910. *Lepus floridanus mearnsi* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 212.
1912. *Sylvilagus floridanus mearnsi* Ruthven and Wood, Proc. Iowa Acad. Sci., vol. 19, p. 204.
1918. *Sylvilagus floridanus mearnsi* Stoner, Iowa Geol. Survey, Bul. No. 5, p. 134.
1921. *Sylvilagus floridanus mearnsi* Gabrielson, Proc. Iowa Acad. Sci., vol. 28, p. 148.

The Mearns Cottontail is found throughout Iowa. It is believed that the cottontail was not very numerous before the land had been opened up by settlement. Land use, as effected by the early settlers, provided a balance of food and cover more suitable to cottontails, and they became more numerous in the vicinity of civilization. Galland (1840, p. 500)

writes: ". . . found in the settled parts of the country," and Allen (1871, p. 194) considers them "Common about the groves and thickets."

The Field Museum of Natural History contains 5 specimens of *mearnsi* from Knoxville, Marion County (Simms, letter, 1936). George G. Goodwin, Assistant Curator, writes of 34 specimens from Iowa City, Johnson County, in the American Museum (letter, 1936).

Published records of museum specimens: Palo Alto Co., Nelson (1909), 8, Museum of Zoology, University of Michigan; Woodbury Co., Sioux City, Nelson (1909), 1, collection of the United States Biological Survey; Van Buren Co., Nelson (1909), 1, collection of the United States Biological Survey; Des Moines Co., Burlington, Nelson (1909), 2, collection of the United States Biological Survey; Polk Co., Fort Des Moines, Nelson (1909), 1, collection of the United States Biological Survey; Johnson Co., Iowa City, Nelson (1909), 3, collection of the United States Biological Survey.

Specimens examined:

Story County, Ames; 1; Iowa State College collection.
Decatur County, Leon; 1; Iowa State College collection.
Appanoose County; 1; Iowa State College collection.
Pottawattamie County; 1; Iowa State College collection.
Fremont County, Sidney; 23a; Iowa State College collection.

(All of the above specimens were referred to *mearnsi* by A. H. Howell.)

Order 7. Artiodactyla

Family Cervidae

AMERICAN ELK
Cervus canadensis canadensis (Erxleben)

1777. [*Cervus elaphus*] *canadensis* Erxleben, Syst. Regni. Anim., vol 1, p. 305.
Type Locality: Eastern Canada.
1871. *Cervus canadensis* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 184.
1883. *Cervus Canadensis* (sic) Goding, Iowa State Agr. Soc. (1882), p. 330.
1890. *Cervus canadensis* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42.
1910. *Cervus canadensis* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 216.

The American Elk was formerly found throughout Iowa, especially common in the northwestern part of the state.

Land use made the extermination of this mammal inevitable, but the blizzard of 1856 did much to hasten the day when elk could no longer be found in the state. Thousands of these mammals were slaughtered while trapped in the deep snow left by the blizzard.

Spurrell (1917) was informed by a hunter that no elk had been seen in Sac County since a herd of 40 passed near Wall Lake in October, 1869. This record for Sac County appears to be the published record for the most recent occurrence of elk in the state. No doubt a few stragglers remained, for the 14th General Assembly extended the closed season in 1872, and in 1898 they were given complete protection by the 27th General Assembly (Bennett, 1926).

PLAINS WHITE-TAILED DEER
Odocoileus virginianus macrourus (Rafinesque)

1817. *Cervus* (misspelled *Corvus*) *macrourus* Rafinesque, American Monthly Magazine, vol. 1, p. 436.
Type Locality: Plains of Kansas River, Upper Mississippi.
1871. *Cervus virginianus* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 186.
1883. *Cervus virginianus* Goding, Iowa State Agr. Soc. (1882), p. 330.
1890. *Cervus virginianus* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42.
1910. *Odocoileus americanus macrouris* (sic) Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 216.

The Plains White-Tailed Deer was formerly found throughout the wooded parts of the state. Great numbers of them were utilized by the early settlers for food and clothing. Galland (1921) writes that hundreds were killed annually. Opening up of the forest provided additional food; hence, the deer populations were temporarily increased or stabilized, but with more intensive land use they were greatly reduced in numbers.

The effect of civilization on the deer population was seen earliest in the eastern part of the state. Fultz (1899) reported them last seen in Muscatine County about 1851. The greatest deer slaughter occurred shortly after the blizzard of 1856. A descriptive picture of the occasion is given by Brainard (1894, p. 393), who writes: "Another lamentable effect of the ice-cap of that winter was the cruel and wanton destruction of wild game. Prior to that season the groves bordering the streams in northern Iowa were well stocked with deer, elk, hare, foxes, wolves, etc. The ice drove these out from sheltering timber to seek food about the farmer's stacks. Men and boys with dogs and guns made savage onslaught upon these. The sharp feet of the larger game cut through the ice and rendered their escape impossible. In some instances they were run down by men on foot, with no other weapon than the family butcher knife, which was all too effective."

In 1856, the 6th General Assembly passed the first law for the protection of deer; the law consisted of a closed season from February 1 to July 15. The numbers of deer may have increased under protection for in 1868 the 12th General Assembly extended the open season by one month Bennett (1926). Allen (1871) considered them more or less common in southwest Iowa during the summer of 1867. The closed season on deer was again lengthened by the 14th General Assembly in 1872 (Bennett, 1926). Mr. V. E. Harris of Oakland informed the writer that his uncle, C. G. Johnson, killed a deer 4 miles southeast of McPaul, Fremont County, in September, 1881, and that the event was considered unusual because deer were thought to have been exterminated in the county. Mosher (1882) reports few remaining in Dickinson County in 1882. Spurrell (1917) writes of one killed in Sac County in 1890. Complete protection was given deer by action of the 27th General Assembly in 1898 (Bennett, 1926).

The race of white-tailed deer living in Iowa today has not been determined. Perhaps they may be partially identified by investigation of the location from which the seed stock was secured. Three distinct herds of captive deer are responsible for the reestablishment of this mammal in Iowa. Mr. William B. Cuppy, of Avoca, Pottawattamie County, possessed

one of these herds. Hon. Frank Beymer, editor of the Avoca Journal-Herald, informed the writer that Cuppy purchased his deer somewhere in Nebraska. Geographic identification would refer Cuppy's deer, supposedly taken in Nebraska, to *Odocoileus v. macrourus.* One night in 1894 the gate to the deer park was opened and all of the deer escaped to the timber on Cuppy's farm. At that time there were 35 in the herd. Twelve years later, during a drive, Beymer estimated their number at 200. Today, withstanding heavy poaching, they are probably no more numerous, but have separated into several herds. On April 15, 1937, Taylor Huston, Iowa Conservation Commission, informed the writer that about 17 deer were to be found north of Hancock, Pottwattamie County, and about 75 northeast of Avoca, and about 46 in the vicinity of Irwin, Shelby County.

A second herd at the Ledges State Park descends from deer formerly kept at the game farm of the old Fish and Game Commission. Mr. Taylor Huston, of the Iowa State Conservation Commission, informed the writer that two of these deer were purchased in Minnesota and that the others were probably taken from those running wild near Avoca. The deer from Minnesota are, by geographic identification, *Odocoileus v. borealis.* A number of deer from the Ledges herd have escaped and are living in the timber along the Des Moines River.

A third herd is located near Keota, Washington County. These deer are known as the Singmaster herd and represent escaped animals which were probably purchased in Nebraska. On April 15, 1937, Taylor Huston, Iowa Conservation Commission, informed the writer that there were about 60 individuals known to be living in that vicinity.

Deer from the three herds discussed have been planted in various parts of the state, and this accounts for those appearing in unusual places.

None of the deer in Iowa have been collected for accurate identification of the subspecies, but it appears that the predominate race is *Odocoileus v. macrourus,* which is the race formerly found over the state.

Family Bovidae

PLAINS BISON
Bison bison bison (Linnaeus)

1758. [*Bos*] *bison* Linnaeus, Syst. Nat. ed. 10, vol. 1, p. 72.
Type Locality: Mexico.
1871. *Bos americanus* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 186.
1883. *Bos Americanus* (sic) Goding, Iowa State Agr. Soc. (1882), p. 330.
1890. *Bos americanus* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42.
1910. *Bison bison* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 216.

The Plains Bison was formerly common over northwest Iowa, straggling into other parts of the state. The location of bison remains by county shows the former range of this mammal very nicely (fig. 1). The work of Pammel (1930) dismisses all doubt as to the former occurrence of this mammal in Iowa.

All of the early reports contain accounts of the bison. Kearny observed 5,000 at Elk Lake in Clay County on July 11, 1820 (Peterson, 1931, p. 302). By 1840 they were occasional on the headwaters of the Des

Moines and Iowa Rivers (Galland, 1921). Early settlers in Sac County considered bison as stragglers after their arrival in 1854 (Spurrell, 1917). Allen (1871) believed it to have been nearly exterminated by the summer of 1867.

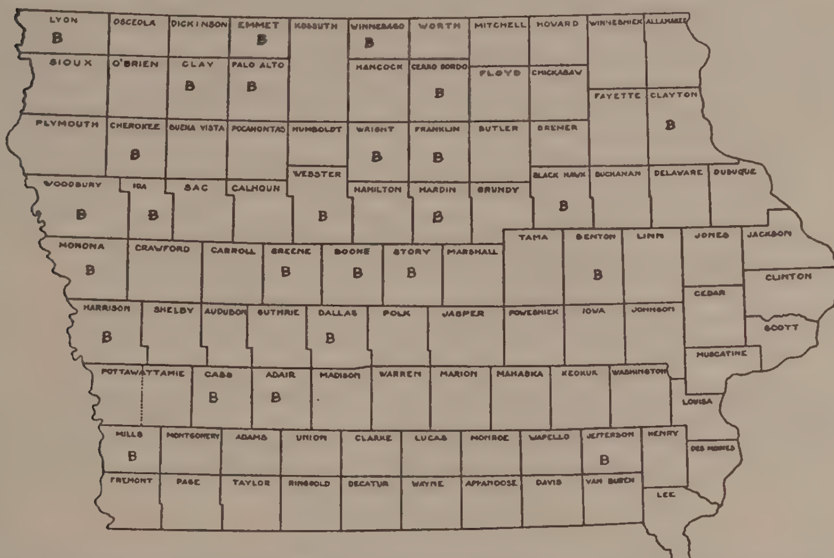

Fig. 1. Counties in which buffalo remains have been found. These records are given on authority of Pammel (1930).

Pammel (1930) writes of a bison killed near Lost Island Lake, Palo Alto County, 1858. One was killed in Boyer Township, Harrison County, in 1864 (Pugsley, 1911). "The Biographical and Historical Record of Greene and Carroll Counties of Iowa" contains a record of a bison shot on June 7, 1864, in Carroll County according to Spurrell (1917).

MAMMALS OF HYPOTHETICAL OCCURRENCE

Order 3. Chiroptera
Family Vespertilionidae

LITTLE GRAY BAT
Myotis grisescens Howell

1909. *Myotis grisescens* Howell, Proc. Biol. Soc. Washington, vol. 22, p. 46.
Type Locality: Nickajack Cave, near Shellmound, Marion County, Tennessee.

With increased investigation this bat may be taken in southern Iowa. Anthony (1928, p. 54) writes that this species is "Found in Tennessee, Missouri and Indiana."

GEORGIAN BAT
Pipestrellus subflavus subflavus (F. Cuvier)

1832. *V. [espertilio] subflavus* Cuvier, Nouv. Ann. Mus. Nat. Paris, vol. 1, p. 17.
Type Locality: Eastern United States.

1871. *Scotophilus georgianus* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 187.
1910. *Pipestrellus subflavus* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 215.

There is small doubt but that the Georgian Bat occurs in eastern Iowa, but existing evidence for including this species is not satisfactory.

Allen (1871) includes this bat on known distribution; Van Hyning and Pellett (1910) fail to offer comment. Miller (1897, p. 91) writes that this subspecies is found in the "Austral zones and casually parts of Transition zone in Eastern United States, from the Atlantic Coast west to Iowa and eastern and southern Texas."

RAFINESQUE BAT
Nycticeius humeralis (Rafinesque)

1818. *Vespertilio humeralis* Rafinesque, Amer. Monthly Mag., vol. 3, p. 445.
Type Locality: Kentucky.
1871. *Nycticejus crepuscularis* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 187.
1890. *Atalapha* (sic) *crepuscularis* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42.

Satisfactory evidence for including the Rafinesque Bat is lacking. It is listed on known distribution by Allen (1871), and Osborn (1890 and 1892) does not offer suitable support for his records.

Order 4. Carnivora
Family Mustelidae

AMERICAN MARTEN
Martes americana americana Turton

1806. [*Mustela*] *americanus* Turton, Linnaeus, System of Nature, vol. 1, p. 60.
Type Locality: Eastern North America.
1883. *Mustela Americana* (sic) Goding, Iowa State Agr. Soc. (1882), p. 330.
1890. *Mustela martes* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42.

The American Marten may have entered northeastern Iowa as a straggler many years ago. This mammal, among the first to retreat before civilization, probably sought its preferred home in the heavy pine and spruce forests at an early date.

The former range, as presented by Seton (1929, vol. 2), passes very near the northeast corner of Iowa. A marten skeleton in the Chicago Academy of Sciences, which is said to have been taken in northern Illinois, is recorded by Cory (1912). This mammal is recorded by Kennicott (1855) for Cook County, Illinois, without comment.

The records for the marten in Iowa by Goding (1883) and Osborn (1890 and 1892) are considered doubtful.

FISHER
Martes pennanti pennanti (Erxleben)

1777. [*Mustela*] *pennanti* Erxleben, Syst. Regni, Anim., vol. 1, p. 470.
Type Locality: Eastern Canada.
1883. *Mustela pennanti* Goding, Iowa State Agr. Soc. (1882), p. 330.
1890. *Mustela pennantii* (sic) Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42.

The Fisher, like the American Marten, may have entered northeastern Iowa as a straggler before and during the days of early settlement.

Van Hyning (1913, p. 311) quotes John G. Smith from the Register and Farmer, Algona: "Plenty of coons and some fishers ran wild in the timber." The species is believed "rare" by Goding (1883). Spurrell (1917) writes of a hunter who supposedly followed fisher tracks in Calhoun County during the late '50's, and of a fur buyer who traded for two fisher skins somewhere north of Sac County. All of these records are subject to question, principally because a description of the animal is not given.

It is of significance that such writers as J. A. Allen (1871), Osborn (1890), Galland (1921) and Mosher (1882) fail to mention this mammal.

NEW YORK WEASEL
Mustela noveboracensis noveboracensis (Emmons)

1840. *Putorius noveboracensis* Emmons, Report Quad. Massachusetts, p. 45.
Type Locality: Southern New York.

This weasel probably enters eastern Iowa and needs only to be collected. Cory (1912, p. 367) issues the following statement in discussing the range of this subspecies: "In the West its range extends at least to the Mississippi River in western Illinois."

COMMON WOLVERINE
Gulo luscus (Linnaeus)

1766. [*Ursus*] *luscus* Linnaeus, Syst. Nat., ed. 12, vol. 1, p. 71.
Type Locality: Hudson Bay.
1883. *Gulo luscus* Goding, Iowa State Agr. Soc. (1882), p. 330.
1890. *Gulo luscus* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42.

The Common Wolverine probably entered northeastern Iowa as a straggler until the middle of the last century.

This species is given a hypothetical rating by Osborn (1890), but in a later publication (1892, p. 5) considers it "Rare or extinct." The wolverine is listed without comment by Goding (1883). It is of significance that Galland (1840) fails to mention this mammal.

A suitable record for the wolverine in 1882 as far south as Knox County, in Indiana, has been reported by Lyon (1936).

LONG-TAILED TEXAS SKUNK
Mephitis mesomelas varians (Gray)

1837. *Mephitis varians* Gray, Charlesworth's Mag. Nat. Hist., vol. 14, p. 581.
Type Locality: Texas.

Mephitis mesomelas varians may be recorded for western and southwestern Iowa. It ranges throughout "Southern and western Texas, eastern New Mexico, and adjacent parts of Mexico; north into Oklahoma, Colorado, Kansas and Nebraska" (Howell, 1901, p. 31). In Nebraska, Swenk (1915, p. 854) considers it "Common over the state."

Family CANIDAE

SWIFT FOX
Vulpes velox velox (Say)

1823. [*Canis*] *velox* Say, Long's Exped. Rocky Mts., vol. 1, p. 487.
Type Locality: South Platte River, Colorado.
1871. *Vulpes velox* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 182.

Vulpes v. velox, a plains species, may have been found in northwestern Iowa before and during the time of settlement. The species is included by Allen (1871) on description. The early settlers in Sac County describe a fox which appears referable to *velox* (Spurrell, 1917). It is given a hypothetical rating by Osborn (1892). The specimen referred to *Vulpes velox* by Nutting (1895) is a misidentification of *regalis*.

Order 5. RODENTIA
Family SCIURIDAE

RUFESCENT WOODCHUCK
Marmota monax rufescens Howell

1914. *Marmota monax rufescens* Howell, Proc. Biol. Soc. Washington, vol. 27, p. 13.
Type Locality: Elk River, Sherburne County, Minnesota.

The Rufescent Woodchuck may straggle into the northern tier of counties in Iowa. The known range includes: "Eastern North Dakota, central and southern Minnesota, Wisconsin, and Michigan, southern Ontario, greater part of New York (including Long Island), and higher parts of western Massachusetts" (A. H. Howell, 1915, p. 25). Surber (1932) is of the opinion that *rufescens* intergrades with *monax* near the Iowa-Minnesota state line.

SOUTHERN GRAY SQUIRREL
Sciurus carolinensis carolinensis Gmelin

1788. *Sciurus carolinensis* Gmelin, Syst. Nat., vol. 1, p. 148.
Type Locality: Carolina.

The Southern Gray Squirrel may straggle into southeastern Iowa. Bennitt and Nagel (1937, p. 24) list this squirrel as "Common to rare throughout Missouri but most numerous in the southeastern third of the state."

Family CASTORIDAE

CANADIAN BEAVER
Castor canadensis canadensis Kuhl

1820. *Castor canadensis* Kuhl. Beitrage z. Zoologie, p. 64.
Type Locality: Hudson Bay.
1871. *Castor fiber* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 190.
1883. *Castor Canadensis* (sic) Goding, Iowa State Agr. Soc. (1882), p. 331.
1890. *Castor fiber* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.

1910. *Castor canadensis* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 214.
1918. *Castor canadensis canadensis* Stoner, Iowa Geol. Survey, Bul. 5, p. 50.

Beavers existing in northeast Iowa today may prove to be of this subspecies. All former publications on beavers in the state have listed the type form, but none may be considered authentic for lack of description and specimens. It is possible that the beaver mentioned for Tama County by Nutting (1892) and for Linn County by Osborn (1905) were *Castor c. canadensis*. No doubt this beaver formerly inhabited the wooded streams of eastern Iowa, but suitable evidence of this occurrence is not available.

Family Cricetidae

LITTLE GRAY HARVEST MOUSE
Reithrodontomys albescens griseus (Bailey)

1905. *Reithrodontomys griseus* Bailey, North Amer. Fauna, No. 25, p. 106.
Type Locality: San Antonio, Bexar County, Texas.

The Little Gray Harvest Mouse may appear in the extreme southwest corner of the state, since the range, according to A. H. Howell (1914) extends to southwestern Iowa.

The record of *griseus* for Palo Alto County by Ruthven and Wood (1912) is a misidentification of *Reithrodontomys megalotis dychei.*

Family Muridae

BLACK RAT
Rattus rattus rattus (Linnaeus)

1758. [*Mus*] *rattus* Linnaeus, Syst. Na., ed. 10, vol. 1, p. 61.
Type Locality: Upsala, Sweden.
1883. *Mus rattus* Goding, Iowa State Agr. Soc. (1882), p. 331.
1890. *Mus rattus* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.
1918. *Rattus rattus* Stoner, Iowa Geol. Survey, Bul. No. 5, p. 68.

There is no authentic record for the Black Rat in Iowa. The species was first listed for the state by Goding (1883) without comment. Osborn (1890) did not know of the species occurring within the state but accepted it on the authority of Jordan. It is probable that these records were largely of opinion and not of fact. Stoner (1918) contributes no additional information.

Family Zapodidae

HUDSON BAY JUMPING MOUSE
Zapus hudsonius hudsonius (Zimmermann)

1780. *Dipus hudsonius* Zimmermann, Geogr. Gesch., vol. 2, p. 358.
Type Locality: Hudson Bay.
1918. *Zapus hudsonius hudsonius* Stoner, Iowa Geol. Survey, Bul. 5, p. 122.

The Hudson Bay Jumping Mouse may come into eastern Iowa, but no authentic record of its appearance has been presented. The status of

this mouse, according to Preble (1899, p. 15) is: "As restricted to the present paper, this species is found from the southern shores of Hudson Bay south to New Jersey, and in the mountains of North Carolina, west to Iowa and Missouri, and northwest to Alaska." Surber (1932) gives the range of this subspecies as over the northern one-half of Minnesota, but records a specimen of *campestris* from Winona County which shows an approach towards *hudsonius*.

The specimens represented by Stoner (1918) as being in the University of Iowa Museum cannot be found (Dill, letter, 1937). Stoner also records specimens in the Coe College Museum. In respect to these specimens, Professor K. A. Stiles writes: "We have two specimens labeled *Zapus hudsonius*, one from Britt, Iowa, collected August 26, 1911, and the other labeled only Iowa." The specimens mentioned by Stoner as having been collected by T. C. Stephens in Dickinson County were not identified beyond the species (Stephens, 1922). Stephens (1922) also writes that the specimen marked "Iowa" in the Coe College Museum was probably collected at the Lakeside Laboratories, Dickinson County.

Family Erethizontidae

CANADA PORCUPINE
Erethizon dorsatum dorsatum (Linnaeus)

1758. [*Hystrix*] *dorsata* Linnaeus, Syst. Nat., ed. 10, vol. 1, p. 57.
Type Locality: Eastern Canada.
1918. *Erithizon* (sic) *dorsatum dorsatum* Stoner, Iowa Geol. Survey, Bul. No. 5, p. 126.

The Canada Porcupine probably straggled into northern Iowa until the period of settlement; however, no suitable records of its presence have been uncovered. Spurrell (1917) was informed by the settlers in Sac County that this mammal was rare on their arrival in 1854.

All records since the time of settlement must be considered accidental, probably escaped pets. Such a record is offered by T. Van Hyning (1913, p. 311), who writes: "In about 1908 'some hounds in the same section,' Allamakee County, 'were badly stuck up by porcupine quills, which had to be pulled from their mouths. Last summer, on French Creek, Allamakee County, I saw some scrub Hemlocks freshly cut and gnawed by porcupines.' Geo. H. Berry, Cedar Rapids, Iowa, April 8, 1913." Dr. George Hendrickson informs the writer of seeing a porcupine taken at Chariton, Lucas County, during the late summer of 1924, but that its captors had not seen evidence of its work in the locality where it was found. The exhibitors of the animal called Hendrickson's attention to an injured forefoot that they assumed to indicate an earlier injury by a trap, and they believed it was an individual from a western state, escaped from a tourist's cage.

The writer observed one taken at Murray, Clarke County, during the summer of '36, and was informed by Taylor Huston, Iowa Conservation Commission, that residents in the community believed the animal to have escaped from a passing tourist's car.

Order 7. Artiodactyla
Family Antilocapridae

AMERICAN PRONGHORN
Antilocapra americana americana (Ord)

1815. *Antilope americana* Ord, Guthrie's Geography, 2d Amer. ed., vol. 2, p. 292 (Described on page 308).
Type Locality: Plains and highlands of the Missouri.

There is no definite proof that the American Pronghorn ever existed in Iowa. It probably entered the northwestern part of the state many years ago. Grinnell (1929), after examining the records of the United States Biological Survey, is led to believe that the antelope was formerly found in western Iowa.

MISINTERPRETATIONS

Order 2. Insectivora
Family Talpidae

STAR-NOSED MOLE
Condylura cristata (Linnaeus)

1758. [*Sorex*] *cristatus* Linnaeus, Syst. Nat., ed. 10, vol. 1, p. 53.
Type Locality: Pennsylvania.
1871. *Condylura cristata* Allen, Proc. Boston Soc. Nat. Hist., vol. 13, p. 187.
1883. *Condylura crestata* (sic) Goding, Iowa State Agr. Soc. (1882), p. 330.
1890. *Condylura cristata* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.

The Star-nosed Mole is not included in the present list because, in 67 years that it has received hypothetical rating in Iowa, no proof of its presence has been uncovered, and, in addition, current evidence does much to eliminate the assumption of its occurrence.

The presence of this mole was assumed in the lists of Allen (1871), Osborn (1890), Goding (1883) and Stephens (1922). Without comment, Osborn (1892) failed to list this species in his second list of Iowa mammals.

The western limit of the known range (Jackson, 1915) for this mole enters extreme northeastern Illinois, the eastern margin and northern half of Wisconsin and northeastern Minnesota. At no point does the range come within less than 115 miles of the northeastern corner of Iowa. Surber (1932, p. 43) provides further evidence againts the probability of this mole's occurrence in Iowa, since he writes: "Living in small colonies it is nowhere common except in the extreme northern and northwestern counties, though occasional examples are taken as far south as the central part of the state."

Fəmily Canidae

EASTERN RED FOX
Vulpes fulva (Desmarest)

1820. *Canis fulvus* Desmarest, Mammalogie, vol. 1, p. 203.
Type Locality: Virginia.

The Eastern Red Fox, *Vulpes fulva,* is found in all former lists of Iowa mammals, but nowhere is it adequately supported by museum specimens. Undoubtedly the correct reference is to *regalis.*

Order 4. Rodentia

Family Sciuridae

BLACK-TAILED PRAIRIE DOG
Cynomys ludovicianus ludovicianus (Ord)

1815. *Arctomys ludovicianus* Ord, Guthrie's Geography, 2d Amer. ed., vol. 2, p. 292. Description, p. 302.
Type Locality: Upper Missouri River.
1883. *Cynomys ludovicianus* Goding, Iowa State Agr. Soc. (1882), p. 331.
1910. *Cynomys ludovicianus* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 214.
1918. *Cynomys ludovicianus ludovicianus* Stoner, Iowa Geol. Survey, Bul. 5, p. 40.

There are a few records of the Black-tailed Prairie Dog in Iowa (Hollister, 1916, and Stoner, 1918). All records of prairie dogs in Iowa are treated as observations of escaped pets. Allen (1871) did not find it east of the Missouri River, and Hollister (1916) considers the 97th meridian the eastern limit through Nebraska.

EASTERN CHIPMUNK
Tamias striatus striatus (Linnaeus)

1758. [*Sciurus*] *striatus* Linnaeus, Syst. Nat., ed. 10, vol. 1, p. 64.
Type Locality: Southeastern United States.
1918. *Tamias striatus striatus* Stoner, Iowa Geol. Survey, Bul. 5, p. 26.

The inclusion of this subspecies by Stoner (1918) is considered a misidentification. Stoner (1918, p. 26) gives the authority upon which the record was based: "A single specimen in the Coe College Museum, collected at Traer, June 23, 1902, and another at Iowa City are the only two definite locality records which are available." There are no specimens of *Tamias* sp. in the Coe College Museum (Stiles, letter, 1937). Cory (1912) refers a specimen examined in the Coe College Museum to *griseus.* The information given is not sufficient to permit tracing of the Iowa City specimen. A statement by A. H. Howell (1929) may account for this misidentification. A. H. Howell (1929, p. 20), referring to *T. s. griseus,* writes: "A large series from Burlington, Iowa, in full summer pelage averages a little deeper ochraceous on the sides of the head and neck, perhaps approaching *T. s. striatus.*" The present range of *T. s. striatus* is "Southeastern United States, from highlands of North Carolina, South Carolina, Georgia and central Alabama west to the Mississippi River in Kentucky and Tennessee; north to the Ohio Valley in Kentucky." (Howell, 1929, p. 14).

Family Cricetidae

SOUTHERN GOLDEN MOUSE

Peromyscus nuttalli aureolus (Audubon and Bachman)

1841. *Mus (Calomys) aureolus* Audubon and Bachman, Proc. Acad. Nat. Sci., Philadelphia, vol. 1, p. 98.
Type Locality: "In the oak forests of South Carolina."
1883. *Hesperomys nuttalli* Goding, Iowa State Agr. Soc. (1882), p. 330.
1890. *Hesperomys aureolus* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.

The Southern Golden Mouse was listed by Goding (1883) without comment. Osborn (1890, p. 43) accepts Goding's record, but concludes: "Not seen, and I think doubtful." The record of *aureolus* is not retained in the present list because there are no specimens for the state, and because the known range of this mouse is considerably to the south of Iowa.

BAILEY WOOD RAT

Neotoma floridana baileyi (Merriam)

1894. *Neotoma baileyi* Merriam, Proc. Biol. Soc. Washington, vol. 9, p. 123.
Type Locality: Valentine, Cherry County, Nebraska.
1883. *Neotoma floridana* Goding, Iowa State Agr. Soc. (1882), p. 331.
1890. *Neotoma floridana* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 43.

The Wood Rat is listed by Goding (1883) without comment. Osborn (1890, p. 43) writes: "One specimen at Iowa Agricultural College, probably taken at Ames." This specimen cannot be found, and the locality record is doubtful.

DRUMMOND MEADOW MOUSE

Microtus drummondi (Audubon and Bachman)

1854. *Arviocola drummondii* Audubon and Bachman, Quadr. N. Amer., vol. 3, p. 166.
Type Locality: Alberta, Canada.
1912. *Microtus drummondi* Ruthven and Wood, Proc. Iowa Acad. Sci., vol. 19, p. 204.

The specimen listed by Ruthven and Wood (1912) has been recently examined by W. H. Burt, Assistant Curator of Mammals of the University of Michigan, and referred to *Microtus p. pennsylvanicus* (letter, 1937). Occurrence of *drummondi* in Iowa would be purely accidental, since Bailey (1900, p. 22) represents the range: "From Hudson Bay to the west slope of the Rocky Mountains and Alaska, and from the northern edge of the United States north to Fort Anderson, N. W. T., in Canadian and Hudsonian zones."

Order 6. Lagomorpha

Family Leporidae

MINNESOTA VARYING HARE

Lepus americanus phaeonotus Allen

1899. *Lepus americanus phaeonotus* Allen, Bull. Amer. Mus. Nat. Hist., vol. 12, p. 11.
Type Locality: Hallock, Kittson County, Minnesota.

1883. *Lepus Americanus* (sic) Goding, Iowa State Agr. Soc. (1882), p. 331.
1910. *Lepus americanus phasonotus* (sic) Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 212.
1918. *Lepus americanus phaeonotus* Stoner, Iowa Geol. Survey, Bul. No. 5, p. 130.

The record by Goding (1883) is given without comment. It is significant that Goding's authority was not accepted by any of the succeeding lists. Van Hyning and Pellett (1910) include the species on the basis of an observation by Pellett. Pellett (letter, 1936) writes: "It is a white rabbit unlike any rabbit common to the state. It was a specimen killed in northern Iowa and in the Museum of Buena Vista College about 1894. Since the record was made a long time later without the specimen for identification, there is a chance that it is mistaken." The inclusion of the species by Stoner (1918) is also based upon this observation by Pellett.

WAGLER'S JACK RABBIT
Lepus callotis Wagler

1830. *Lepus callotis* Wagler, Nat. Syst. der Amphibien, p. 23.
Type Locality: Southern end of Mexican table-land.
1883. *Lepus callotis* Goding, Iowa State Agr. Soc. (1882), p. 331.
1890. *Lepus callotis* Osborn, Proc. Iowa Acad. Sci., vol. 1, p. 42.

This species is first listed by Goding (1883). Osborn (1890, p. 42) follows Goding but considers the record "Very doubtful." There is no specimen of this species from the state, and its occurence here could only be accidental.

GREAT PLAINS JACK RABBIT
Lepus californicus melanotis (Mearns)

1885. *Lepus californicus texianus* True, Proc. U. S. Nat. Mus., vol. 7 (1884), p. 601.
Type Locality: Independence, Montgomery County, Kansas.
1918. *Lepus californicus melanotis* Stoner, Iowa Geol. Survey, Bul. 5, p. 133.

Stoner (1918) includes this jack rabbit on the basis of a specimen taken in Johnson County during the autumn of 1915. This specimen cannot be located and its occurrence in the state can, at best, only be considered accidental.

Order 7. Artiodactyla
Family Cervidae

COMMON MOOSE
Alces americana americana (Clinton)

1822. *Cervus americanus* Clinton, Letters on Nat. Hist. and Int. Resources of New York, p. 193.
Type Locality: "Country north of Whitestown," New York.
1910. *Alces americanus* Van Hyning and Pellett, Proc. Iowa Acad. Sci., vol. 17, p. 216.

There is no suitable evidence that the Common Moose was ever present in Iowa. Van Hyning and Pellett (1910, p. 216) write: "The only record known of this species in Iowa is the finding of several teeth in the Boone Mound; supposing they were inhabitants of the territory and were used as food by the prehistoric natives."

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CONTENTS

Abstracts of Doctoral Theses

NOTE: Complete copies of these theses may be consulted at the Library, Iowa State College, Ames, Iowa.

CHEMICAL INVESTIGATIONS OF THE EFFECT OF FERTILIZER RATIOS AND GREEN MANURES ON YIELDS AND COMPOSITION OF CROPS AND THE ORGANIC MATTER IN NORFOLK SAND[1]

James Edward Adams

From the Department of Chemistry, Iowa State College

Accepted for publication June 28, 1937

The experiments reported constitute a part of a general study of green manure and fertilizer problems associated with the fertility of sandy soils of the South. They were conducted under the auspices of the Bureau of Plant Industry of the United States Department of Agriculture, in co-operation with the South Carolina Agricultural Branch Station (the Sandhill Experiment Station) near Columbia, South Carolina.

The sandhill area extends from central North Carolina, across South Carolina and Georgia, and a short distance into Alabama. The soils are principally of the Norfolk and related series; the sandhill phase of Norfolk sand, on which the experiments were conducted, is of a coarse and open texture. The native cover, following deforestation of the long-leaf pine, is principally dwarf oak, sedge, and other plants capable of growing in soil low in fertility and subject to excessive leaching. The soil is classified as a yellow podsol, and contains but a small amount of organic matter of a wide carbon: nitrogen ratio. The importance of the conservation of nitrogen is at once apparent. That portion of the area which lies in South Carolina varies from 10 to 30 miles in width, and comprises approximately 10 per cent of the area of the State. The cultivated areas are used principally for the production of corn and cotton; more recently peaches, grapes, dewberries and asparagus are being grown.

EXPERIMENT NO. 1—A GREEN MANURE FERTILIZER STUDY

Green manuring is an accepted practice for the addition of organic matter and nitrogen to the soil. The effect on the fertility of the soil will depend on climatic conditions and the character of the soil, so the turning of forage crops to the soil may be an uneconomic practice. In order to study this, 24 plats (one-twentieth acre), arranged in 6 tiers of 4 plats each, were used in a rotation of legumes, corn and cotton; the tiers were paired for the sake of comparison. The legumes, that, is, soybeans, velvet beans, and cowpeas, were grown on one tier with 400 pounds of a 6-8-4 fertilizer[2], and on the second with a like application of 2-8-4 mixture. An additional plat of cowpeas, to be followed by a winter cover of rye and vetch, completed each of the two tiers. The hay was removed from the tier receiving the 6-8-4 fertilizer, and the stubble was turned; the full crop was turned on the 2-8-4 tier during September of each year. The same ratios were maintained on the proper tiers for the corn and cotton which followed in the rotation; the corn received 400 pounds and the cotton 800 pounds of fertilizer, respectively.

[1] Original thesis submitted June, 1936. Doctoral thesis number 383.

[2] The fertilizer analysis is N — P_2O_5 — K_2O.

The average data obtained for the six years of the experiment allow the following conclusions to be drawn: (1) larger yields of all crops were obtained by applying a 6-8-4 fertilizer to the stubble of the legumes than by the use of the 2-8-4 mixture, all plats being left fallow during the winter; (2) the use of rye and vetch as a winter cover increased yields over those of the respective winter-fallow plats, and again the higher-nitrogen analysis produced the greater yields; (3) on fallow plats the 6-8-4 combination was more efficacious in conserving organic carbon, but following a winter cover there was no particular difference; (4) there was a considerable reduction in the carbon: nitrogen ratio, which is thought to be associated with an increased availability of the soil nitrogen, and (5) there was but a loose correlation between the yields of corn and cotton and the carbon content of the plats at the end of the second rotation.

Supplementing and adding to the information given in Experiment No. 1, was a combination experiment to study the effect of 21 fertilizers on the composition of soybean hay and seed, and that of the crop residues on the organic matter and pH of the soil. The triangle system, as applied to fertilizer investigations by Schreiner and Skinner[3], was used. Each of the 21 plats was subdivided into 3 subplats: (1) from one of the subplats the seed was harvested and the threshed hay returned to the proper plat, (2) another furnished hay samples for analysis and the crop was turned as a green manure, and (3) the hay was removed from the remaining subplat and the stubble turned.

EXPERIMENT NO. 2—THE EFFECT OF FERTILIZERS ON THE YIELD AND COMPOSITION OF SOYBEAN HAY

The following conclusions were drawn: (1) the best average yield was obtained with a fertilizer containing nitrogen, phosphoric acid and potash in the ratio 1:3:1 (3-9-3 analysis), (2) the calcium content of the hay is in proportion to the superphosphate content of the fertilizer, (3) the nitrogen content is related to that of the calcium, (4) calcium and potassium present an inverse relation, with the former appearing as the "key element," and (5) the phosphate content is a reflection of both the potash and the superphosphate contents of the fertilizer. The variations in soluble ash, Mn_3O_4, R_2O_3, MgO, and sulphur in the hay are also discussed.

EXPERIMENT NO. 3—THE EFFECT OF FERTILIZERS ON THE YIELD AND COMPOSITION OF SOYBEAN SEED

The data showed the following: (1) a small amount of nitrogen is needed for seed production, but a definite need for phosphate is displayed, (2) the factors controlling the nitrogen content of the hay also operate in the seed, and (3) nitrogen and potash are needed to induce the highest oil content in the seed.

EXPERIMENT NO. 4—THE EFFECT OF FERTILIZERS AND CROP MANAGEMENT ON THE ORGANIC MATTER AND pH OF THE SOIL

The three managements of the crop residues in conjunction with the fertilizer treatment allowed a study of the combined effects on the carbon and nitrogen relations, and the pH of Norfolk sand. The following obser-

[3] Schreiner, O., and Skinner, J. J. Bot. Gaz. 50:1-30. 1910, and Jour. Amer. Soc. Agron. 14:193-197. 1918.

vations are pertinent: (1) a gain in organic carbon was obtained on one of the 69 subplats; this was with the 3-9-3 analysis, (2) both gains and losses of nitrogen occurred; the particular result obtained depended on the management of the residues and the fertilizer used, (3) a rapid narrowing of the carbon: nitrogen ratio occurred early in the experiment, followed by a gradual decrease. This would seem to indicate that a portion of the native organic matter is rapidly utilizable by micro-organisms, and (4) the pH of the soil is also amenable to both the type of residue turned and the fertilizer employed; decreases were most numerous, but the use of certain combinations resulted in increases.

SUMMARY

The results obtained over a period of six years indicate that, under the climatic conditions of the Southeast, a departure from some generally accepted agricultural practices is necessary. A soil subject to excessive leaching and aeration, in conjunction with a fairly high level of temperature and rainfall, does not allow an efficient usage of summer legumes turned as green manures. It is better to remove the hay crop and apply liberal quantities of nitrogen to the summer crops. Winter cover crops appear to have a definite place in the management of Norfolk sand.

The management of a soil containing organic matter of a wide carbon: nitrogen ratio should involve practices conducive to a lowering of this ratio, to induce a more labile nitrogen content.

Fertilizers affect materially the composition of soybean hay grown on this type of soil. The data submitted from 21 fertilizers of varying analysis indicate that the amount of calcium supplied in the fertilizer dominates the nutrition of the soybean plant.

The pH of Norfolk sand reflects the management of the crop residues and the fertilizer used to produce the crop. Fertilizers high in potash tend to maintain, or increase, the pH of this soil. Appreciable decreases were produced with certain combinations.

THYSANOPTERA OF IOWA[1]

FLOYD ANDRE

From the Department of Zoology and Entomology, Iowa State College

Accepted for publication June 28, 1937

The first systematic study of Iowa Thysanoptera was published by Miss Alice Beach (1895)[2] in a paper which recorded 13 species for the state. Seven of this number have since been shown to be synonyms of other species. Moulton and Andre (1936)[3] recorded 85 species for the state, 4 of which were described as new to science.

This paper brings together 87 species of Thysanoptera which have been recorded as occurring in the state and contains keys for the separation of the various species A study of the food plants from which the species are ordinarily collected has been made.

During the winter months the writer collected leaf mold, moss, grass, dead laves and other debris from many places in the state to obtain a knowledge of the forms normally overwintering in Iowa as well as the habitat usually serving this purpose. Collections made in this manner were unusually productive and many interesting and rare as well as several new species were discovered. A number of species were very restricted as to their ecological habitat and could be collected in but a few limited places.

The general characters of the Thysanoptera are discussed. Sexual characters of the two suborders are taken up, as well as the general distribution and importance of this order from the standpoint of Iowa and other sections of the country. Species of particular economic significance in Iowa are *Thrips tabaci* Lindeman, *Taeniothrips simplex* (Morison) and *Dendothrips ornatus* (Jablon.).

This is followed by the general classification of the Thysanoptera and a description of the various genera and species other than is given in the keys separating the various divisions. A complete list of references of all the pertinent papers dealing with Iowa species is given at the end of the thesis.

The following 48 species belonging to the suborder *Terebrantia* Haliday are recorded from the state: *Aeolothrips albicinctus* Haliday, *Aleolothrips bicolor* Hinds, *Aeolothrips fasciatus* (Linné), *Aeolothrips nasturtii* Jones, *Heterothrips arisaemae* Hood, *Heliothrips haemorrhoidalis* (Bouché), *Hercothrips fasciapennis* (Hinds), *Hercothrips femoralis* (Reuter), *Echinothrips americanus* Morgan, *Chirothrips manicatus* Haliday, *Chirothrips obesus* Hinds, *Limothrips cerealium* Haliday, *Limothrips denticornis* Haliday, *Aptinothrips rufus* (Gmelin), *Sericothrips apicalis* Hood, *Sericothrips beachae* Hood, *Sericothrtps cingulatus* Hinds, *Sericothrips interruptus* Hood, *Sericothrips sambuci* Hood, *Sericothrips tiliae* Hood, *Sericothrips variabilis* (Beach), *Dendothrips ornatus* Jablonowski,

[1] Original thesis submitted December, 1936. Doctoral thesis number 410.

[2] Beach, Proc. Iowa Acad. Sci., 3: 214-227 (1895).

[3] Moulton and Andre, Iowa State State College Jour Sci., 10: 223-234 (1936).

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The following 39 species of the suborder *Tubulifera* Haliday were found in Iowa: *Cryptothrips rectangularis* Hood, *Cephalothrips elegans* Moulton, *Cephalothrips errans* Moulton, *Hoplothrips americanus* (Hood), *Hoplothrips angusticeps* (Hood), *Hoplothrips flavicauda* (Morgan), *Hoplothrips flavus* Moulton and Andre, *Hoplothrips pergandei* (Hood), *Hoplothrips quercus* Moulton and Andre, *Hoplothrips smithi* (Hood), *Eurythrips flavacinctus* Moulton and Andre, *Eurythrips osborni* Hinds, *Eurythrips tarsalis* Hood, *Liothrips caryae* (Fitch), *Liothrips citricornis* (Hood), *Liothrips leucognis* Hood, *Liothrips ocellatus* Hood, *Liothrips sambuci* Hood, *Rhynchothrips pruni* Hood, *Lissothrips muscorum* Hood, *Neothrips corticis* Hood, *Allothrips nubillicauda* Watson, *Allothrips megacephalus* Hood, *Haplothrips aculeatus* (Fabr.), *Haplothrips faurei* Hood, *Haplothrips graminis* Hood, *Haplothrips leucanthemi* (Schrank), *Bagnalliella yuccae* (Hinds), *Karnyothrips flavipes* (Jones), *Leptothrips mali* (Fitch), *Neoheegeria verbasci* (Osborn), *Glyptothrips flavascens* Hood, *Phloeothrips (Hoplandrothrips) xanthopus* Hood, *Phloeothrips (Acanthothrips) nodicornis* Reuter, *Neurothrips magnafemoralis* (Hinds), *Bolothrips bicolor* (Heeger), *Elaphrothrips armatus* (Hood), *Elaphrothrips flavipes* (Hood), and *Elaphrothrips tuberculatus* (Hood).

OXIDATION, REDUCTION AND HYDROLYSIS OF WOOL KERATIN[1]

MILDRED BARR

From the Department of Chemistry, Iowa State College

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This study was undertaken to obtain quantitative data of the change in composition and mechanical strength of wool keratin brought about by hydrochloric acid, sodium chloride, steam, sodium hydrosulfite, and potassium permanganate.

Hydrochloric acid. Five grams of wool keratin, prepared from plain-woven undyed wool by extraction with water and ether, were immersed in 125 cc. of water, 0.10 *N*, 0.25 *N*, 0.50 *N*, 1.00 *N*, 6.87 *N*, or 7.87 *N* hydrochloric acid for ten hours at 25° C. or in 250 cc. of water, 0.25 *N*, 0.50 *N*, or 0.75 *N* hydrochloric acid in a flask fitted with a water-cooled reflux condenser for one hour at 100° C.; the residual keratins were washed with water until the rinse gave no test for chloride before determination of weight, nitrogen (3), total sulfur (2), wet strength, and elongation (1). Dilute hydrochloric acid in ten hours at 25° C. had but slight effect on the weight, total sulfur, total nitrogen, wet strength, and elongation of wool; concentrated hydrochloric acid in ten hours at 25° C. brought about appreciable decrease in the weight and total nitrogen, approximately 50 per cent decrease in the strength and only slight decrease in the total sulfur of wool; degradation of the keratin by hydrochloric acid in one hour at 100° C. increased with increasing concentration of acid, the wet strength decreased more rapidly than either weight or nitrogen, and the total sulfur remained almost unchanged. The residual wool slowly decreased in nitrogen and increased in sulfur.

Sodium chloride. Five grams of the wool keratin were treated with 250 cc. of 0.06 *M* or 0.70 *M* sodium chloride for one hour at 100° C. and then washed with distilled water until the rinse gave no test for chloride. The residual keratin differed little from the original in weight, nitrogen, total sulfur, and wet strength.

Steam. Five grams of the keratin were steamed for one hour at 100°, 115.2°, 121°, 126°, 134.5°, and 141.5° C. and for one, three and five hours at 115.2° C in an autoclave; the residual keratin was washed free of soluble products by eight rinsings in water before determination of weight, nitrogen, total sulfur, and wet strength. The degradation of the keratin by steam increased with increasing pressure or time; the first appreciable loss of both sulfur and nitrogen occurred at 134.5° C. in one hour and at 115.2° C. in five hours. The weight, total sulfur, and nitrogen of the wool decreased more slowly than its wet strength which was only sixteen per cent of the original after one hour at 126° C.

Sodium hydrosulfite. The wool keratin was immersed in fifty volumes of 0.9525 *M* sodium hydrosulfite for ten hours at 40°C. and then washed until the rinse no longer decolorized permanganate. The residual

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wool analyzed higher in nitrogen and total sulfur and slightly lower in sulfate sulfur but retained only fifty per cent of its original wet strength.

Potassium permanganate. The wool keratin was immersed for ten hours at 40° C. in fifty volumes of 0.01 *M*, 0.15 *M*, 0.02 *M*, 0.03 *M*, or 0.04 *M* potassium permanganate, in fifty volumes of 0.01 *M*, 0.015 *M*, 0.02 *M*, 0.03 *M*, 0.04 *M*, 0.05 *M*, or 0.06 *M* potassium permanganate made 0.18 *N* with sulfuric acid, and in 62.5, 75, 87.5, or 100 volumes of 0.02 *M* potassium permanganate or 0.02 *M* potassium permanganate made 0.18 *N* with sulfuric acid. The residues were freed from oxides of manganese by 0.5 per cent sodium hydrosulfite in two hours at room temperature, washed in water until the rinse no longer decolorized permanganate and then analyzed for weight, total nitrogen, total sulfur, sulfate sulfur (4), and wet strength. The strength of the wool decreased much more rapidly in the aqueous than in the acidic solution and the weight, total nitrogen, and non-sulfate sulfur decreased less rapidly than the strength and to the same extent in aqueous and acidic solutions. Percentage losses of weight, nitrogen, and non-sulfate sulfur were quite similar, indicating solution of wool at the surface of the fiber rather than elimination of a portion containing a different ratio of sulfur to nitrogen. The weight, nitrogen, non-sulfate sulfur, and wet strength of wool decreased with increasing volume of 0.02 *M* aqueous and acidic potassium permanganate; the decrease in strength was more rapid in the aqueous than in the acidic solution. Although residual keratins from treatment with other oxidants have been reported to contain sulfate equivalent to part of their original sulfur, in no case in this study was the sulfate sulfur of wool increased by potassium permanganate; solution of part of the original (0.93 per cent) sulfate sulfur occurred in the acidic potassium permanganate. The residual keratin from treatment with fifty volues of 0.06 *M* acidic potassium permanganate for ten hours at 40° C. yielded 0.48 per cent and the blank 0.01 per cent ash. Although aqueous solutions of potassium permanganate are more commonly used in the processing of wool, acidic solutions have been shown more desirable and a specification of volume as well as concentration of oxidant necessary.

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THE REDUCTION OF FURAN COMPOUNDS AND BIOLOGICAL STUDIES OF THE PRODUCTS OF REDUCTION[1]

CHESTER WAYNE BRADLEY

From the Department of Chemistry, Iowa State College

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The compounds studied were conveniently placed into two groups: (1) furan and derivatives; and (2) dibenzofuran and derivatives. An effort was made to obtain partially reduced compounds from the reductions.

I. FURAN COMPOUNDS

A survey of the reported work on the reduction of furan and derivatives disclosed that the only instance of a dihydrofuran derivative resulting from the reduction of a furan derivative was the production of dihydrodehydromucic acid from dihydromucic acid by means of sodium amalgam. The attempted partial hydrogenation of β-furylacrolein led to no isolable products.

Furan was hydrogenated in the presence of a platinum oxide catalyst in acetic acid and dioxan solvents to yield tetrahydrofuran and n-butyl alcohol. Partial hydrogenation yielded the same products and unchanged furan. Hydrogenation of furan in the presence of Raney nickel catalyst and in dioxan or decalin solvent or without a solvent yielded only tetrahydrofuran. 2-Methylfuran was partially hydrogenated in the presence of Raney catalyst to 2-methyltetrahydrofuran. 2,5-Dimethylfuran was partially hydrogenated under the same conditions but the products could not be separtaed by fractional distillation.

The rates of hydrogenation of furan and dihydrofuran under various conditions of catalyst and solvent were determined and compared graphically. In every case dihydrofuran was hydrogenated more rapidly. Additional interesting facts concerning the stability of the furan ring were brought out by the determinations of the rates of hydrogenation. All of the curves were smooth, indicating that the hydrogenations were not stepwise. With the platinum oxide catalyst, furan in dioxan absorbed three moles of hydrogen to give a smooth hydrogenation curve. This indicated that the furan ring was cleaved. Under the same conditions dihydrofuran absorbed only one mole of hydrogen, indicating that the dihydrofuran ring and the resultant tetrahydrofuran ring were not cleaved. The hydrogenations were not complete in n-hexane solvent but dihydrofuran did absorb more than one mole of hydrogen showing that either the dihydrofuran or tetrahydrofuran ring was cleaved.

II. DIBENZOFURAN COMPOUNDS

Previous to this investigation dibenzofuran had been reduced noncatalytically to 1,2,3,4-tetrahydrofuran and catalytically to perhydrodibenzofuran. No attempt had been made to reduce dibenzofuran deriva-

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tives. In this investigation dibenzofuran and some amino and hydroxy dibenzofurans were subjected to catalytic hydrogenation at both low and high pressures and to reduction by sodium in liquid ammonia.

Dibenzofuran could be made to yield only perhydrodibenzofuran by catalytic reduction. It was subjected to hydrogenation in acetic acid solution in the presence of platinum oxide and platinum black catalysts and to hydrogenation at high pressure and temperature in the presence of Raney nickel catalyst.

2-Hydroxydibenzofuran and 4-hydroxydibenzofuran in acetic acid solution were hydrogenated in the presence of platinum oxide catalyst to absorb three moles of hydrogen. Only perhydrodibenzofuran was obtained. 2-Methoxydibenzofuran was then tried under similar conditions to yield an inseparable mixture of products.

Hydrogenation of 4-hydroxydibenzofuran at 140 degrees and 70 atmospheres in the presence of a nickel catalyst also yielded some perihydrodibenzofuran. The remainder was alkali soluble but could not be purified. It probably was a mixture of unreduced and reduced 4-hydroxydibenzofuran. 4-Methoxydibenzofuran was then tried under similar conditions except that dioxan solvent was used. In this case much less perhydrodibenzofuran was formed and 41 per cent of 1,2,3,4-tetrahydro-6-methoxydibenzofuran was obtained by fractional distillation. It crystallized from petroleum ether, m.p., 39-39.5 degrees.

New, and in some cases improved, syntheses of aminodibenzofurans were employed. 3-Nitrodibenzofuran suspended in ethanol was hydrogenated at 100 degrees and three atmospheres pressure employing Raney nickel catalyst. Yields of 90-95 per cent of 3-aminodibenzofuran of high purity were obtained. 2-Bromodibenzofuran and 4-bromodibenzofuran were subjected to the action of sodamide in liquid ammonia to obtain the corresponding amines.

3-Aminodibenzofuran and 4-aminodibenzofuran were dissolved in dioxan and subjected to hydrogenation at 235 degrees and 170 atmospheres for three hours. A nickel catalyst was employed. No appreciable hydrogenation occurred. 3-Aminodibenzofuran in ethanol was hydrogenated at 3 atmospheres and 100 degrees, employing Raney catalyst. The hydrogenation was very low. A dihydroaminodibenzofuran was produced m. p. 72 degrees. It did not form a salt with moist carbon dioxide indicating that the reduction was in the non-substituted ring. The reduced amine formed a dibromide, m.p., 186 degrees, with decomposition. 3-Aminodibenzofuran was regenerated by the action of alcoholic potassium hydroxide on the dibromide.

By the action of sodium in liquid ammonia dibenzofuran was reduced to 1,4-dihydrodibenzofuran, m. p. 43 degrees. 4-Hydroxydibenzofuran was reduced to 1,4-dihydro-6-hydroxydibenzofuran, m. p. 116-117 degrees. The methyl ether (m.p., 54 degrees) was prepared by the use of methyl sulfate. 4-Methoxydibenzofuran yielded 26 per cent of dihydrodibenzofuran. 3-Aminodibenzofuran, 3-diethylaminodibenzofuran and 4-aminodibenzofuran reacted with sodium in liquid ammonia yielding tars and humus-like materials as the only product. Approximately three-fourths of the amines were recovered. Calcium in liquid ammonia reduced dibenzofuran to a greater extent than did sodium in liquid ammonia. The stage of the reduction was not so specific as the product was a mixture.

Dihydrodibenzofuran was subjected to the action of several reagents. It was reduced to tetrahydrodibenzofuran by sodium and alcohol and catalytically in the presence of either platinum oxide or Raney catalysts. It was oxidized to dibenzofuran by permanganate, dichromate, hypochlorous acid and, in small amounts, by ozone. Bromine was added readily to yield a dibromide, m.p., 79 degrees. Acetylation by means of acetic anhydride and stannic chloride yielded 1,4-dihydro-7-acetyldibenzofuran, m.p., 117 degrees.

Metalation of dihydrodibenzofuran was of especial interest because it offered the best evidence of its structure. Metalation with *n*-butyllithium at low temperature followed by carbonation produced a dihydrodibenzofurancarboxylic acid (m.p., 278-279 degrees), which was dehydrogenated to 2-dibenzofurancarboxylic acid. A similar reaction with 1,4-dihydronaphthalene yielded 1,2-dihydronaphthalene 2-carboxylic acid which was dehydrogenated to β-naphthoic acid.

Lithium reacted with dibenzofuran in ether or dioxan solution to give *o*-hydroxybiphenyl. Dihydrodibenzofuran in dioxan also reacted with lithium to give *o*-hydroxybiphenyl, dehydrogenation having occurred during the reaction.

SYNTHESIS OF CERTAIN FURAN AND DIFURYL DERIVATIVES[1]

ELLIS VINCENT BROWN

From the Department of Chemistry, Iowa State College

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This thesis has for its purpose the extension of our knowledge concerning the furan series of compounds. The first section concerns the oxidation of furan methyl groups and certain other furan compounds; the second deals with the bromination of furyl methyl ketone; the third concerns the synthesis of several new members of the difuryl series.

I. OXIDATION OF FURAN METHYL GROUPS

Although oxidation in the furan series has been studied since 1873, when Limpricht (1) worked with furoic acid, no general method and few special methods have been reported for the oxidation of furan compounds without decomposition of the furan ring. A short history of oxidation in the furan series is given.

The oxidizing agent used in these studies is one that has been applied successfully by W. A. Noyes (2) to the benzene series, and appears to be general for the oxidation of furan methyl groups to carboxylic acids. This reagent, potassium ferricyanide, combines power with mildness in a way which makes it an ideal oxidizing agent in the furan series. Table 1 gives the results of a number of oxidations.

II. BROMINATION OF FURYL METHYL KETONE

The great tendency of furan to undergo nuclear substitution when treated with a reagent which can effect furan nuclear substitution has been demonstrated in the case of ethyl furylacrylate (3) and 2-furyl phenyl ketone (4). The only exception has been the addition of bromine to the side chain of furyl ethylene (5). In the bromination of furyl methyl ketone it has been shown that the bromine enters the side chain to give ω-bromofuryl methyl ketone. This behavior may be explained by the theory of C. F. Ward (6). This theory assumes enolization of the carbonyl group and addition to the unsaturated linkage with a final removal of hydrogen bromide. It was expected, however, that the second atom of bromine would substitute in the nucleus. This was shown not to be the case as the dibromination of furyl methyl ketone gives ω,ω-dibromfuryl methyl ketone in 90 per cent yield.

The nitration product of furyl methyl ketone (7) has been shown to be 5-nitro-2-furyl methyl ketone by comparison with the product from the reaction of diazomethane with 5-nitro-2-furfural. This ketone has been characterized by the preparation of the oxime.

III. SYNTHESES IN THE DIFURYL SERIES

It is significant that, although a large number of diphenyl compounds and many phenylated heterocyclic compounds have been prepared, very

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TABLE 1

Compounds oxidized	Sample grams	Grams potassium ferricy-anide used	Acid obtained	Yield grams
Sylvan	1	50	furoic	.05
Dimethyl furan	1	25	dehydromucic	.01
Furyl methyl ketone	1	25	furoic	.51
Furylacrylic acid	1	25	not oxidized	
5-Methyl-2-furoic	1	25	dehydromucic	.35
Furfural	1	25	furoic	.22
5-Bromofuryl methyl ketone	1	25	5-bromofuroic	.45
Furyl methyl ketone	1	25	furoic	.30
5-Nitrosylvan	1	25	5-nitrofuroic	.54
Furfuryl alcohol	1	25	furoic	.21
Furfural acetone	1	25	furylacrylic	.10
Furfural acetone	1	25	furylacrylic	.10
Furil	½	25	furoic	.41
2-Methyl-2-furoic acid	1	75	2,3-dicarboxy furan	.35
Tertiarybutyl furoic acid	½	50	dehydromucic	.01
Furyl ethylene	1	25	furoic acid	.02

little work has been published concerning the difuryl series of compounds. A review of the possible methods of preparation of difuryls is given. Table 2 lists in chronological order all of the difuryls known at the present time.

Unsuccessful attempts were made to couple 5-bromo-2-furyl methyl ketone, ethyl 5-chloro-2-furoate, ethyl 5-bromo-2-methyl-3-furoate, and 2,5-dimethyl-3-iodofuran.

One particular object in the studies on the difuryl types was to prepare a compound which would show optical activity. The method of Stanley and Adams (8) for determining which diphenyls would be resolvable was applied to the difuryl series. After a thorough consideration of all the resolvable difuryls possible the only one that seemed to offer a chance for successful preparation was 2,2′,5,5′-tetramethyl-3,3,-difuryl-4,4′-dicarboxylic acid. This compound was prepared. Although optical studies on this compound are not completed, the results should throw some very interesting light on the structure of the furan ring.

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TABLE 2

Compound	Method used	Source	Reference
5,5′-dinitro-2,2′-difuryl		Furan	Ann. chim. phys., (8) 4,233 (1905)
3-carboethoxy-2,2′-difuryl	Ring closure	Ethyl furoyl acetate	J. Pharm. Soc. Japan, 544,501 (1927)
2,2′-difuryl-3-carboxylic acid	Saponification	3-Carboethoxy-2,2′-difuryl	Ditto
2,2′-difuryl	Decarboxylation	2,2′-difuryl-3-carboxylic acid	Ditto
5-furyl-2-furfuraldehyde	Gatterman	2,2′-difuryl	Helv. Chim. Acta, 15,1066 (1932)
5-furyl-2-furoic acid	Oxidation	5-furyl-2-furfural	Ditto
5,5′-diodo-2,2′-difuryl	Coupling of RMgx	5-iodo-2-furylmagnesium iodide	Wright, Doctoral Thesis, Iowa State College. 1932.
5,5′-dicarboethoxyl-2,2′-difuryl and acid	Coupling Cu	Ethyl 5-bromo-2-furoate	This Thesis
5,5′-dimethyl-2,2-dicarbomethoxy-4,4′-difuryl and acid	Coupling Cu	Methyl 5-methyl-4-iodo-2-furoate	This Thesis
2,2′,5,5′-tetramethyl-3,3′dicarboethxy-4,4′-difuryl and acid	Coupling Cu	Ethyl 2,5-dimethyl-4-iodo-3-furoate	This Thesis

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FRACTIONATION OF OAT HULL LIGNIN[1]

WILBUR SCHEIRICH CLAUS

From the Department of Chemistry, Iowa State College

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The difficulty of repeating another investigator's research on lignin has been a well known fact for many years. Two different investigators working on the same lignin-containing tissue and using the same methods have often been unable to reproduce each other's work. Extensive discussions on the difficulty of repeating another investigator's work have been reported by Aberhalden, Fuchs, Freundenberg, and Phillips.

It was necessary in this investigation to repeat some of the work begun by Walde. The ammonia lignins isolated by the same method but by two different investigators in this laboratory have been found to agree in their oxidation values and their carbon and hydrogen analyses. The methoxyl content of the two prepared lignins differed by 1.6 per cent.

The fact that the ammonia lignin prepared from oat hulls could be fractionated into an acetone soluble and an acetone insoluble fraction indicated that the ammonia lignin, as previously reported, was not a homogeneous product. These two fractions differed considerably. From the analyses of the products methylated with diazomethane and dimethyl sulfate the number of hydroxyl and methoxyl groups could be readily calculated.

The seeming unreliability of methylating with dimethyl sulfate and caustic in studies on the constitution of lignin has been pointed out by Compton and Hibbert. High temperatures and excess alkali during the process of methylation seem to cause the formation of new hydroxyl groups, which are in turn methylated. Compton and Hibbert have therefore suggested (a) the use of acetone as a solvent; (b) a slight excess of alkali (5-10 per cent); and (c) a temperature of 20° C. as the best conditions for methylation. With the omission of the use of acetone as a solvent, these precautions were used when lignin derivatives were methylated with dimethyl sulfate and caustic. For the calculations it was assumed that the methylation with dimethyl sulfate and caustic, under these carefully controlled conditions, gave products which were completely methylated.

It was found in this study that the acetone insoluble fraction of the ammonia lignin when methylated with diazomethane gave a product having the same methoxyl content as a product which had been methylated with dimethyl sulfate and caustic. This fact would indicate that the latter method of methylation has not brought about any drastic changes in the molecule during the methylation process. The acetone insoluble fraction was found to have three methoxyl and three hydroxyl groups as compared to three methoxyl and five hydroxyl groups in the acetone soluble fraction.

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The NaOI and NaOBr oxidation of the acetone soluble and insoluble fraction of the ammonia lignin were, respectively: acetone soluble, 212.0, 240.0; acetone insoluble, 163.0, 226.0. It was of interest to note that iodoform could be isolated from the alkaline iodine oxidation of both the acetone soluble and insoluble fractions, indicating that the grouping from which it arose had not been destroyed during the extraction process.

The iodo-carboxy lignin prepared from the ammonia lignin was found to differ considerably from the iodo-carboxy lignin prepared from the acid-hydrolyzed oat hulls. The former had an average minimum molecular weight of 960, whereas the latter had an average minimum molecular weight of 1,340. From the analyses of the iodo-carboxy lignin prepared from the ammonia lignin and its derivatives it is not at all unlikely that this isolated lignin is a mixture which could be readily fractionated with suitable solvents.

It was observed that the iodo-carboxy lignin prepared from the ammonia lignin which had been fully methylated by means of dimethyl sulfate and caustic would not go completely into solution when treated with hot 10 per cent NaOH. This phenomenon was in contradicition to the result obtained by Walde. Both the alkali soluble and insoluble fractions of the methylated derivative showed losses in methoxyl content. It would appear from these results that the fully methylated iodo-carboxy lignin prepared from the ammonia lignin is a mixture which is separated into distinct fractions by treatment with hot 10 per cent NaOH.

The fact that the iodo-carboxy lignin prepared from the acid-hydrolyzed oat hulls could be quantitatively oxidized with NaOI resulting in the formation of reoxidized lignins was of note. The once oxidized iodo-carboxy lignin had four hydroxyl and two methoxyl groups; the same number as the twice oxidized iodo-carboxy lignin. Iodoform was the product isolated from the oxidation in each case.

The iodo-carboxy lignin prepared from the acid-hydrolyzed oat hulls was fractionated by the use of acetone which yielded hitherto unreported acetone soluble and insoluble fractions. A 10 per cent increase in weight of the two fractions beyond the weight of the original oxidized lignin seemed to indicate that the acetone was in some manner reacting with the oxidized lignin. It was most unlikely that this increase in weight of these two fractions was due to an absorption or occulsion phenomenon.

It was found that the acetone soluble fraction of the above described oxidized lignin had five hydroxyl and one methoxyl groups, while the acetone insoluble fraction had five hydroxyl and three methoxyl groups. Of the five hydroxyl groups in the acetone insoluble fraction, two were shown to be carboxylic in character. The fully methylated derivatives of the acetone insoluble fraction were completely soluble in hot 10 per cent NaOH with a definite loss in methoxyl content. These soluble derivatives when re-methylated with diazomethane and again treated with hot 10 per cent NaOH failed to go completely into solution under any set of circumstances.

The acetone insoluble fraction of the iodo-carboxy lignin prepared from the acid-hydrolyzed oat hulls was re-oxidized with NaOI to give an oxidized lignin having four hydroxyl and two methoxyl groups. The same number of hydroxyl and methoxyl groups was observed on the re-oxidized iodo-carboxy lignins which would indicate that by repeated oxidations an oxidized product common to the lignin molecule is obtained.

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THE EFFECT OF A GAS FILM ON METAL SURFACES USED FOR ELECTRON RECORDING[1]

M. ALDEN COUNTRYMAN

From the Department of Physics, Iowa State College

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In 1930 Dr. P. H. Carr announced a new method of recording electrons on a number of metals by means of the altered surface activity produced by exposure to electrons of moderate velocity.

His results and those of a number of other experimenters have resulted in the presentation of four more or less conflicting theories of electron recording on metals. They are:

1. That the electron stream cleans the surface to some extent of its surface gas film, and thus alters its activity toward corroding vapours.
2. That the electron stream cleans the surface of the thin film of fatty acid always present on surfaces cleaned in the ordinary manner.
3. That the exposure to electrons accelerates the formation of a thin layer of the oxide which renders that portion of the surface "passive" toward chemical vapours.
4. That the electron stream causes the formation on the surface of a film of organic polymers from organic vapours present.

THE PROBLEM

This research was undertaken in an effort to determine if possible which one or ones of the above suggested explanations most nearly covered all the facts of electron recording.

APPARATUS AND EXPERIMENTAL METHOD

The metal used as the object of investigation in this experiment was platinum, chosen because of its high melting point, its inertness, and because the electron record on platinum can be developed in mercury vapor. By including in the tube system a small amount of liquid mercury it was possible to expose the strip and develop the resulting record without removal from the high vacuum conditions. During exposures the mercury vapour pressure in the tube resulting from the presence of this liquid mercury was reduced to a negligible value by submerging the tube in a thermos bottle filled with CO_2 in acetone.

The electron camera consisted of an untreated tungsten filament, a copper plate-grid combination, and the platinum strip all sealed within a pyrex tube of convenient size to fit into a large-mouthed thermos bottle as previously mentioned. The platinum strip was supported on separately insulated leads so that it could be heated by an electric current for outgassing. This electrical isolation also made it possible to locate the electron beam accurately on the strip by simply noting the electron current to the

[1] Original thesis submitted August, 1936. Doctoral thesis number 401.

strip and adjusting the deflecting field until this electron current was a maximum.

Permanent records of each exposure were secured by photographing the strip through the walls of the tube by means of a suitably arranged camera and light.

The vacuum system contained no wax or grease joints, being one continuous glass system from the electron camera through the trap, and the mercury vapour pump to the stop-cock in the line to the fore-pump. The tube proper was baked out for long periods at approximately 450° C. and the remainder of the system and the trap were flamed out at intervals during the series of tests.

Preliminary tests with a McLeod gauge sealed into the system showed that a vacuum of better than .02 micron (the limit of the gauge used) could consistently be reached. In fact, the vacuum was high enough to cause the mercury in the McLeod gauge to adhere to the top of the closed column until the mercury level in the open leg fell more than five centimeters below its level.

RESULTS AND DISCUSSION

Well defined electron records were secured when there was present on the platinum strip a film resulting from exposure to dry air, commercial nitrogen, or commercial hydrogen, even after a cursory baking out and evacuation.

No records were secured if the strip had been heated to 1200° C. and the system had been baked out several times at a temperature of 450° C., or if the strip had been exposed to commercial argon gas and then merely evacuated before exposure to electrons.

In the case of commercial nitrogen gas anomalous records were secured consisting of a grey line on a dark background instead of the usual dark line on a grey background characteristic of records with dry air or commercial hydrogen.

In one instance, however, after nitrogen had been evacuated for only a brief period before exposure, the record secured was the usual dark line on a grey background. This was the only time nitrogen acted in what one is tempted to call the normal manner for electron recording.

All exposures were to electrons of some 90 volts equivalent velocity and the electron current was usually about 50 microamperes. Exposure times were generally two hours and a half, although some were as long as four hours. The difference in strength of impression was not marked and it was assumed that all the exposures were well over the minimum required to produce a record.

CONCLUSIONS

The author believes the results obtained warrant the following conclusions:

1. The process of electron recording is complex but in general results from the fact that the effect of an electron exposure is to produce, in the exposed region, all of the constituents in the atomic state, so that any combination of the elements involved is possible. The direction in which the resulting reactions will proceed depends upon the equivalent concen-

trations of the reacting substances, and the stability and concentration of the end-products produced.

2. Electron recording on platinum is definitely not due to a surface change in the metal itself, but does depend upon the presence of an extremely minute amount of some foreign material, most probably a residual gas film from certain gases.

3. If there is present on the surface a gas film which is in quasi-chemical combination with the surface atoms of the metal, but which does not form stable compounds with the metal, the effect of the electron exposure will be to remove this gas film where the electrons have struck and thus render the surface more active at that region.

4. If the gas is one which may form stable compounds with the metal then the effect of the electrons will be to facilitate the formation of that compound and render the surface more passive at that point.

5. The fact that electron recording may occur in the absence of water and/or grease vapour indicates that it does not depend solely upon these agencies. However, in their presence the production of organic polymers whose low vapour pressures would effectively eliminate them from the reacting region, is entirely possible. On the other hand, if oxygen were present the resulting oxidation would probably result in the removal from the region of organic vapours in the form of CO_2.

6. No evidence was secured as to the removal by the electrons of a residual film of grease in the manner indicated by some of the recent investigators. The occurrence of organic polymers as a result of exposure to moderate velocity electrons would tend to suggest that such cleaning would not take place unless oxygen is present. However, in the light of the considerable evidence gathered by other workers in this and the related field of "breath figures" it seems unwise to conclude that such cleaning might not take place.

HIGH MOLECULAR WEIGHT FATTY ACID DERIVATIVES[1]

GROVER M. FORD

From the Department of Chemistry, Iowa State College

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Although derivatives of the high molecular weight fatty acids are rather numerous, there are very few described in the literature which might be entirely satisfactory for identifying the acids from capric to stearic. Of the derivatives that have been described, there is considerable discrepancy in the melting points as reported by different observers. Progress in the identification of natural products and in the analysis of artificial mixtures depends largely on the possibility of preparing suitable crystalline derivatives of the substances being investigated.

A series of derivatives for identifying lauric, myristic, palmitic, stearic and oleic acids has been prepared. The melting points of some of these derivatives are recorded in table 1.

TABLE 1. *Melting points of derivatives of lauric, myristic, palmitic, stearic and oleic acids*

Derivatives	Lauric	Myristic	Palmitic	Stearic	Oleic
N-Acylcarbazole	78–79°	81–82°	85–86°	91–92°	oil
N-Acylphenothiazine	70°	75°	80°	86°	oil
N-Acyl-p-toluenesulfonamides	83–84°	89–90°	93–94°	98–99°	oil
p-Phenylphenacyl esters	86°	90°	94°	97°	60°
p-Nitroanilides	78°	84°	93°	96°	....
N-Acylsaccharin	88–89°	90–91°	90°	95°	oil
2,4-Dinitrophenylhydrazides	110–111°	118°	120–121°	123°	oil
N-Acyl-2-nitro-p-toluidines	62–63°	73–74°	78–79°	85°	....
p-Tolylmercuric salts	93–94°	95–96°	99°	102–103°	oil
Phenylmercuric salts	82°	86°	93°	95°	oil
Triphenyllead salts	91°	102–103°	110°	112°	....
Monoureides	182°	178°	175°	174°	160°
Monothioureides	138°	135°	135–136°	133°	113°
p-Xenylamides	146°	143°	142°	143°	....
p-Diphenyl ketones	101–102°	102–103°	103–104°	106–107°	....
2,8-Diacylcarbazole	176°	169°	162°	163°	....
p-Acylaminobenzoic acids	227–228°	224–225°	226–227°	221°	....
Diacylbenzidine	248°	241–242°	233°	232°	....

The carbazole, phenothiazine and 2-nitro-*p*-toluidine derivatives, the *p*-toluenesulfonamides, *p*-nitroanilides and *p*-xenylamides were all prepared by heating the acid chlorides with carbazole, phenothiazine, 2-nitro-*p*-toluidine, *p*-toluenesulfonamide, *p*-nitroaniline and *p*-xenylamine, respectively, from 100-160° without a solvent. Palmitoylcarbazole has been reported[2]. The *p*-phenylphenacyl esters were prepared from *p*-phenylphenacyl bromide and the sodium salt of the acids. These esters of lauric,

[1] Original thesis submitted March, 1937. Doctoral thesis number 418.

[2] Copisarow, *J. Chem. Soc.*, 113, 816 (1918).

stearic and oleic acids have been reported[3]. The saccharin derivatives were prepared by refluxing the sodium salt of saccharin and the acid chlorides in dry chloroform. The 2,4-dinitrophenylhydrazides were prepared by refluxing the base and the acid chlorides in dry benzene[4]. The phenylmercuric and *p*-tolylmercuric salts were prepared by refluxing diphenylmercury and di-*p*-tolylmercury with the acids in xylene.

The triphenyllead salts were prepared by refluxing tetraphenyllead and the acids in xylene. Tetraphenyltin under the same conditions gave no reaction.

The monoureides and monothioureides were prepared by refluxing the acid chlorides with urea and thiourea in anhydrous pyridine. The monoureides of stearic and oleic acids have been reported[5]. The diphenyl ketones were prepared by the Friedel-Crafts reaction in dry carbon disulfide. The 2,8-diacylcarbazole derivatives were prepared by means of the Friedel-Crafts reaction in dry nitrobenzene. The *p*-acylaminobenzoic acids were prepared by refluxing *p*-aminobenzoic acid and the acid chlorides in anhydrous pyridine. The diacylbenzidine derivatives were prepared by refluxing two equivalents of the acid chlorides with benzidine in dry pyridine.

Distearoylthiourea (m.p. 100°) was prepared from ethyl stearate, sodium ethylate, thiourea and a few cubic centimeters of pyridine. N-Palmitoylanthranilic acid (m.p. 100°) and N-stearoylanthranilic acid (m.p. 113°) were prepared by refluxing for five hours a mixture of anthranilic acid and the acid chlorides in dry chloroform. 3-Palmitoylaminodibenzofuran (m.p. 130°) and 3-stearoylaminodibenzofuran (m.p. 134°) were prepared by heating together equimolecular quantities of the acid chlorides and 3-aminodibenzofuran without a solvent.

2-Stearoylcarbazole (m.p. 103-105°) was prepared by the Friedel-Crafts reaction from stearoyl chloride and carbazole in dry nitrobenzene. 2-Stearoylcarbazole when heated with stearoyl chloride gave N-stearoyl-2-stearoylcarbazole (m.p. 87°). Hydrolysis of N-stearoyl-2-stearoylcarbazole yielded stearic acid and 2-stearoylcarbazole.

Attempts to mercurate stearone and ethyl stearate resulted in failures. Mercuration of stearonitrile with mercuric acetate in glacial acetic acid gave stearamide in seventy per cent yields. Attempted reaction of stearone with metallic sodium resulted in a quantitative recovery of stearone.

The ten best series of derivatives in this study may be listed in the following order of decreasing importance: (1) N-acylcarbazole derivatives; (2) N-acyl-*p*-toluensulfonamides; (3) *p*-phenylphenacyl esters; (4) N-acylphenothiazine derivatives; (5) N-acyl-2-nitro-*p*-toluidine derivatives; (6) N-acyl-saccharin derivatives; (7) 2,4-dinitrophenylhydrazides; (8) *p*-nitroanilides; (9) phenylmercuric salts; (10) *p*-xenylamides.

No very definite conclusions can be drawn concerning melting points and chemical constitution. The N-acyl types of derivatives seem to be the most satisfactory, especially when the nitrogen is a part of a ring system. However, the *p*-phenylphenacyl esters are very satisfactory derivatives of lauric, myristic, palmitic, stearic and oleic acids.

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[3] Drake and Bronitsky, *J. Am. Chem. Soc.*, 52, 3715 (1930).

[4] Cerezo and Olay, *Anales soc. espan. fis, quim.*, 32, 1090 (1934).

[5] Stendel, *Comp. rend.*, 196, 1810 (1933); (b) Jacobson, *J. Am. Chem. Soc.*, 58, 1984 (1936).

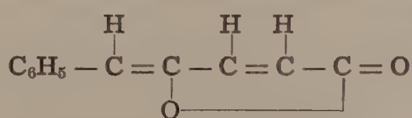
REARRANGEMENTS IN THE FURAN SERIES[1]

Raymond A. Franz

From the Department of Chemistry, Iowa State College

Accepted for publication June 28, 1937

The instability of compounds in the furan series has for many years been recognized as a serious factor in the successful investigation of this branch of organic chemistry. As a natural outgrowth of this phenomenon, the problem arose concerning the structures of certain decomposition resins. It was found by Hewlett[2] that the reaction between 5-chloro- or 5-bromo-2-furfural and phenylmagnesium bromide produced unstable compounds which were erroneously designated as carbinols. They were found to contain halogens and upon standing decomposed. Hydrogen chloride and hydrogen bromide were evolved respectively, and the same stable, halogen-free compound was produced in each case. This work was repeated by Wright[3], who obtained identical results. In some unpublished work[4] he later reduced the final halogen-free compound with hydriodic acid to phenyllevulinic acid, and also oxidized it to maleic anhydride and benzoic acid. On this basis the structure assigned was the lactone of *gamma*-hydroxy-*delta*-phenyl-*alpha, gamma*-pentadienoic acid, m.p. 85°;



As a continuation of this work the author postulated an ether structure for this unstable intermediate and found that its molecular weight was approximately double that of the carbinol. Furthermore, a Zerewitinoff analysis showed that no active hydrogen was present. In view of this latter fact, a number of analyses were made on various known carbinols such as furfuryl alcohol, furylphenylcarbinol, and diphenylfurylcarbinol. Each of these showed approximately one active hydrogen. In order to prove the presence and position of the hydroxyl group in a compound of this type, furylphenylcarbinol was oxidized and simultaneously nitrated, producing known 5-nitro-2-furylphenyl ketone. These facts led to the conclusion that the Grignard reagent had reacted normally with furfural and ethyl furoate and consequently should react normally with halogen-substituted furan analogues.

Hewlett reported that the Grignard-complex from 5-chloro-2-furfural and phenylmagnesium bromide was hydrolyzed with acetic acid and then subjected to steam distillation until a slight darkening occurred. It was

[1] Original thesis submitted December, 1936. Doctoral thesis number 408.

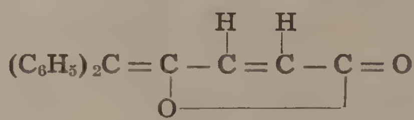
[2] A. P. Hewlett, Doctoral Thesis, "Furfural and Some of Its Derivatives," Library, Iowa State College, p. 83 (1930).

[3] G. F. Wright, Doctoral Thesis, "Furfural and Some of Its Derivatives," Library, Iowa State College, p. 62 (1932).

[4] G. F. Wright, Unpublished Work, Iowa State College, 1936.

found that when the Grignard-complex was hydrolyzed with ammonium chloride instead of acetic acid, no darkening was noticed upon prolonged steam distillation. If one drop of dilute acetic acid was added, however, an immediate discoloration developed and the unstable, intermediate, halogen-containing ether was obtained. This showed that a distinct chemical change had taken place because of the presence of acetic acid, hence the true carbinol had been overlooked. To further substantiate this conclusion, (5-chloro-2-furyl)phenylcarbinol was prepared in *n*-butyl ether solution and an aliquot sample was dried prior to a Zerewitinoff analysis which showed the presence of a carbinol. Since this carbinol was extremely unstable and could not be purified by the customary methods, the above unusual procedure for its analysis was justified.

The major portion of the thesis deals with the attempted preparation of tertiary-halogeno-furyphenyl carbinols and their subsequent decomposition products. The first compound of this type was prepared from ethyl 5-bromo-2-furoate and phenylmagnesium bromide. The carbinol was found to be extremely unstable, loosing hydrogen bromide with such ease that its decomposition sometimes became violent. Consequently, intermediates could not be found, and only a stable, halogen-free lactone, m.p. 111°, was isolated. Its structure was designated as the lactone of *gamma*-hydroxy-*delta,delta*-diphenyl-*alpha, gamma*-pentadienoic acid;



The same compound was produced when ethyl 5-chloro-2-furoate was treated with the Grignard reagent.

The structure of this lactone was proved by degradation methods in several ways. First, the reduction with hydriodic acid in a sealed tube produced *delta,delta*-diphenyllevulinic acid, m.p. 107°-108°. Secondly, hydrolysis with sodium carbonate, followed by reduction with zinc dust, again produced diphenyllevulinic acid. Since this acid was not described in the literature, its structure was proved both by synthesis and degradation.

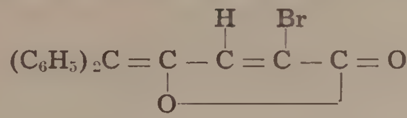
Chichibabin[5] made *delta*-phenyllevulinic acid by the catalytic scission of the furan ring in furylphenylcarbinol. Diphenylfurylcarbinol was treated in a similar manner and yielded diphenyllevulinic acid. Cleavage of diphenyllevulinic acid with alcoholic potassium hydroxide yielded diphenylmethane and succinic acid.

The third step in the proof of structure for the lactone was the action of alcoholic potassium hydroxide, which produced diphenylmethane and fumaric acid. Lastly, it was found that dilute aqueous sodium hydroxide yielded *asymm*-diphenylacetone. All degradation products were identified by appropriate derivatives.

A number of other mono- and dihalogen-substituted lactones were synthesized from the corresponding di- and trihalogenofuroic esters and the Grignard reagent. One of these produced from ethyl 4,5-dibromo-2-furoate and phenylmagnesium bromide was found to contain a halogen

[5] Chichibabin, *Chimi and industrie,* **11,** 563 (1931), Congres de Chimie.

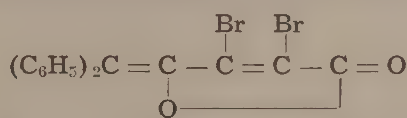
atom and was designated as the lactone of *alpha*-bromo-*gamma*-hydroxy-*delta,delta*-diphenyl-*alpha,gamma*-pentadienoic acid, m.p. 158°;



Upon reduction with hydriodic acid, diphenyllevulinic acid was obtained. Dilute sodium hydroxide produced in addition to *asymm*-diphenylacetone, oxalic acid and diphenylacetic acid. Since *asymm*-diphenylacetone could not possibly have been formed unless the bromine atom had been attached to the *alpha*-carbon in the lactone, then the original furoic ester must have been 4,5-dibromo- and not 3,5-dibromo- as Hill[6] reported.

Further evidence for the 4,5- positions of the two bromine atoms in dibromofuroic acid was offered by first preparing what Hill called *alpha*-anilido-crotonolactone from the above dibromo-acid. Besides this, there was prepared *beta*-anilido-crotonolactone from tetronic acid which was synthesized by two different methods reported in the literature[7]. A mixed melting point of Hill's compound with the other *beta*-lactone proved their identities and also showed that Hill's material was a *beta*-derivative and not *alpha*-anilido-crotonolactone.

Since (5-bromo-2-furyl) diphenylcarbinol was never isolated for more than a few seconds because of its extreme instability, the proof that it was a true carbinol was still lacking. Hence diphenylfurylcarbinol was brominated and after decomposition was complete, a small amount of *alpha,-beta*-dibromo-*gamma*-diphenylmethylene-crotonolactone, m.p. 211°;



together with considerable quantities of the mono-bromo-lactone were isolated. Both of these lactones were also synthesized by the action of the Grignard reagent on the corresponding di- and tri-halogeno-furoic esters, the decomposition proceeding through the unstable carbinols.

Hale and co-workers[8] have reported that diphenylfurylcarbinol on standing for a short time gradually melted into a gummy, reddish-brown mass, but did nothing further with this material. It was found, however, that upon trituration with acetone, this resinous material yielded a white solid whose physical constants suggested a bis (diphenylfurylmethyl) ether formula, m.p. 215° (dec). Its structure was established by the simultaneous hydrolysis and splitting of the furan ring to diphenyllevulinic acid. Furthermore, this ether, when brominated, decomposed and yielded the same *alpha*-bromo-lactone as had been prepared by the action of phenylmagnesium bromide on ethyl 4,5-dibromo-2-furoate. This series

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[6] Hill and Sanger, *Proc. Am. Acad. Arts Sci.*, **21**, 159 (1885-1886).

[7] Wolff and Schwabe, *Ann.* **291**, 231 (1896), Anschütz and Bertram, *Ber.*, **36**, 469 (1903).

[8] Hale, McNally and Pater, *Am. Chem. J.*, **38**, FR, (1906).

of reactions tends to justify an ether structure for the unstable halogen-containing compound which Hewlett obtained.

SUMMARY

1. The reaction of 5-halogeno-2-furoic esters and 5-halogeno-2-furfural with phenylmagnesium bromide has been found to be a normal one.

2. The intermediate compound formed by the reaction of 5-halogeno-2-furfural with phenylmagnesium bromide has been found to be a bis (5-halogeno-2-furylphenylmethyl) ether.

3. These halogenofurylcarbinols are exteremely unstable and lose halogen acids with ease, giving substituted crotonolactones.

4. These lactones have been identified by degradation methods.

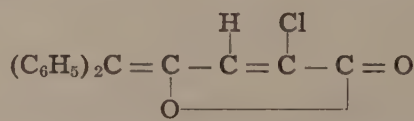
5. Further proof has been given for the structure of Hill's dihalogenofuroic acids.

6. Several mechanisms have been offered for the decomposition of 5-halogeno-2-furylarylcarbinols.

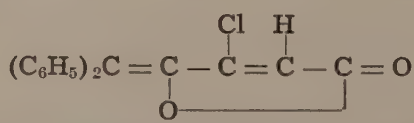
7. Those news compounds not mentioned above are as follows:

(a) (5-Chloro-2-furyl) dimethylcarbinol, m.p. 42°.

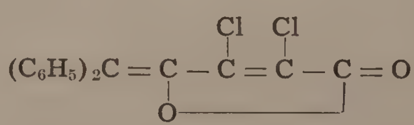
(b) *alpha*-Chloro-*gamma*-diphenylmethylene-crotonolactone, m. p. 127°;



(c) *beta*-Chloro-*gamma*-diphenylmethylene-crotonolactone, m.p. 128.5°;



(d) *alpha*, *beta*-Dichloro-*gamma*-diphenylmethylene-crotonolactone, m. p. 178°;



THE PHALAENIDAE OF MISSISSIPPI—MORPHOLOGY OF THE GENITALIA[1]

Ross E. Hutchins

From the Department of Zoology and Entomology, Iowa State College

Accepted for publication June 28, 1937

The object of the present study is to illustrate and describe the male genitalia of the Phalaendae (Noctuidae) of Mississippi in such a manner as to render their identification comparatively simple. At this time 108 genera and 182 species have been recorded from the state. The species included here represent all the major groups of this large and widely distributed family and, thus, give a "bird's eye view" of the genital structures found in the entire family.

The moths upon which the work is based were collected chiefly at light traps which have been operated at various places in the state for more than six years. Prior to the time that the author began collecting moths in Mississippi the late F. H. Benjamin did a great deal of collecting there. Thus it is thought that but few additional species remain to be collected except perhaps in the semi-tropical Gulf Coast area.

The terminology employed for the various genital structures is that of McDunnough. This is the system that has been adopted by almost all modern lepidopterists. The order of arrangement follows the Barnes and McDunnough check list.

In studying the genitalia of these moths it is often difficult to determine with certainty the homology of the parts in the different species. Specialization among the Phalaenidae has brought about not only modification of the various parts but in many cases brought about complete loss of the entire structures. In some instances the process of modification seems to have affected the two sides of the genitalia differently so that asymmetry results.

The work, of which this is an abstract, although dealing chiefly with the genital structures, is actually a monograph of the species found within the borders of Mississippi. For this reason all available data on hosts and collection records have been included.

In this abstract only a short summary of the genital structures found in the various genera is given since space prohibits a consideration of all the species.

DISCUSSION OF THE MALE GENITALIA OF THE GENERA

Heliothis. Genitalia simple, generalized; claspers narrow, no armature except a corona.

Rhodophora. Structure similar to *Heliothis,* except for a stronger sacculus and a well developed harpe.

Lygranthoecia. Genitalia hardly distinguishable from those of *Rhodophora.* Apex of clasper slightly more obtuse, harpe slightly longer.

Schinia. Similar to *Rhodophora.*

[1] Original thesis submitted July, 1936. Doctoral thesis number 391.

Feltia. More varied than in preceding genera. Claspers broad, margins sub-parallel; corona a straight row of spines; harpe long, slender, curving, uncus slender.

Agrotis. Represented by two species. *A. ypsilon* Rott. appears very close to *Feltia* group while *A. c-nigrum* L. has a pollex, a short, obtuse harpe and is otherwise quite different.

Epipsilia . Claspers drawn to an acute apex; harpe short, broad, obtuse; uncus spatulate.

Lycophotia. Clasper with apex rounded, more or less excavated on ventral margin before apex; harpe slender; uncus slender with apex upturned.

Polia. Clasper broad, greatly excavated before apex on ventral margin; ampulla long, curved; harpe flap-like.

Eriopyga. Clasper narrower in distal portion, apex rounded; harpe strong, well developed; uncus weak.

Nephelodes. Clasper with apex broad, excurved, pollex present; harpe broad, apex obtuse; uncus broad, spatulate.

Morrisonia. Clasper with apical margin straight; pollex claw-like, set on ventral angle; harpe slender, falcate; uncus slender.

Orthosia. Clasper, broad, deeply excavated on ventral margin, before apex; pollex greatly produced, slender; harpe slender, falcate; sacculus well developed; uncus with apex diamond-shaped.

Xanthopastes. Clasper broad, thick, apex rounded, left member bearing a harpe; harpe broad, trigonate; uncus rounded, thickened.

Cirphis. This genus exhibits a marked deviation from the typical Phalaenid type. Clasper with a distinct constriction near center; sacculus broad, expanded.

Borolia. Similar to *Cirphis.*

Neleucania. Clasper greatly excavated on ventral margin before apex, pollex short, claw-like; harpe short, slender; apex of succulus extended over base of harpe.

Cucullia. Genitalia generalized, clasper with margins sub-parallel; harpe short, apex obtuse.

Psaphida. Clasper broad, margins sub-parallel; harpe arising near ventral margin; uncus slender.

Eutolype. Clasper moderately slender; harpe short, obtuse.

Conistra. Clasper with a slender process from costal angle; harpe with apex clavate.

Amphipyra. Clasper expanded in distal portion, apex rounded; armature absent.

Dipterygia. Clasper with apex rounded, pollex present; harpe short, weak; uncus slightly clavate.

Trachea. Clasper broad, armature weak or absent.

Perigea. Clasper moderate, curving; harpe strong; uncus usually weak.

Callopistria. Clasper reduced, apex slender; peniculus well developed, uncus stout.

Acherdoa. Clasper without armature, apex narrower.

Chytonix. Clasper gently incurved, peniculus, angulate, uncus expanded.

Harrisimemna. Clasper short, broad; harpe excurved, stout.

Polygrammate. Clasper slender distally; ampulla present; harpe strong, excurved.

Leuconycta. Clasper ovate, broad; harpe long, weak; uncus weak.

Acronycta. Sacculus well developed, apex usually produced into three processes of variable shape; uncus weak.

Delta. Pollex present; sacculus well developed; clavus denticulate.

Catabena. Sacculus with apex bifid, editum produced.

Prodenia. Sacculus greatly developed, nearly as large as clasper; harpe arising in distal region.

Laphygma. Similar to *Prodenia.*

Caradrina. Clasper long and broad, a denticulate area before ventral angle.

Gulgula. Sacculus produced beyond ventral angle, apical portion of clasper inturned, uncus short, stout.

Crambodes. Clasper broad, ovate; harpe moderately developed, uncus weak.

Platysenta. Clasper slender, curved, corona absent, uncus cygnate.

Monodes. Clasper incurved, corona obsolescent; peniculus well developed; harpe variable.

Apamea. Apical portion of clasper distinct, trigonate; peniculus and uncus weak.

Achatodes. Apical portion of clasper rounded; harpe short, broad.

Pyrrhia. Clasper slender, apex slightly larger; uncus weak.

Papaipema. Pollex greatly produced, spinate; harpe stout, excurved.

Ogdoconta. Clasper expanded apically, sacculus narrow, free.

Cosmia. Clasper simple, without armature.

Stiria. Clasper broad, apex rounded; harpe from ventral region.

Stiriodes. Clasper ovate; harpe short, clavate.

Plagiomimicus. Clasper gently tapering toward apex; sacculus moderate.

Amolita. Clasper with apex expanded and divided into two parts.

Euthisanotia. Apex of clasper acute; sacculus with apex slender and free.

Cydosia. Clasper constricted near center; harpe stout, excurved. Left clasper larger.

Phobolosia. Clasper simple; harpe a rounded prominence.

Amyna. Clasper with costal margin produced near base, armature obsolescent.

Chamyris. Apex of clasper produced; apex of sacculus greatly produced, incurved.

Lithacodia. Clasper gently incurved; harpe short, curved.

Xanthoptera. Clasper narrower in apical portion, two trigonate processes from disto-costal margin.

Cryphia. Clasper weak, extreme apex slightly expanded; sacculus greatly developed, apex produced.

Helicontia and *Spragueia.* Slightly asymmetrical. Costal angle slightly produced; sacculus strong, apex sometimes free; uncus mandibulate.

Tarachidia and *Tarache.* Clasper simple; sacculus strong; harpe moderate, incurved.

Marathyssa. Ventral angle greatly produced; costal angle slightly produced; armature obsolescent.

Paectes. Clasper short, broad; sacculus strong; apex free; a stout, conical process from uncus base.

Baileya. Clasper with a narrow costal process arising near base; harpe from ventral area.

Catocala. Claspers mostly moderate; apex often produced; armature simple or complex; uncus manidbulate.

Allotria. Slightly asymmetrical; clasper narrower in apical region; armature weak.

Caenurgia. Costal angle bearing a process; other armature absent or greatly developed.

Pelamia. Ampulla foot-like; harpe short, weak; sacculus strong.

Phurys. Ampulla stout, excurved; uncus enlarged near center.

Celiptera. Clasper slender; ampull stout, excurved.

Argyrostrotis and *Zale.* Usually asymmetrical; armature complicated and greatly developed; clasper more or less reduced.

Charadra. Clasper short, broad, a long costal process near base; uncus short, broad.

Matigramma Asymmetrical; sacculus well developed; apex free in left member.

Autographa and *Plusia.* Clasper moderately slender; armature variable; uncus often considerably produced.

Raphia. Harpe short, ovate; sacculus weak.

Strenoloma. Apex of clasper expanded, costal margin thickened and produced; uncus short, tri-lobate.

Melipotis. Clasper ovate; armature weak; uncus short, bearing a claw.

Phoberia. Clasper narrow; harpe short, stout; uncus short, bearing a claw.

Anticarsia . Clasper ovate; armature obsolescent.

Litoprospus. Harpe arising near ventral margin, slender; sacculus weak.

Panopoda. Clasper moderate, apex rounded, no armature, a scent pouch from outside surface, uncus cygnate.

Bendis. Clasper narrower toward apex, sacculus strong; armature variable.

Noropsis. Asymmetrical. Left clasper broader, bearing a pollex, other armature absent.

Erebus. Clasper short, apex narrow, uncus cygnate.

Epizeuxis, Scoleocampa, Tetenolita, Palthis. Clasper narrow, divided in apical region into several processes, sacculus usually strong, apex often free.

Phiprospus. Clasper narrow, armature weak.

Phalaenostola, Hormisa, Dercetis. Clasper narrow, apex with costal or ventral or both angles produced, other armature absent.

Bomolocha. Clasper short, broad, armature obsolescent; uncus short, falcate.

Metalestra. Editum greatly produced, spinate, apex of clasper slender.

Plusiodonta. Clasper short, broad, a broad process from ventral angle; apical portion of sacculus thickly denticulate.

Rivula. Clasper ovate, armature obsolescent.

Anomis. Similar to *Rivula* but bearing scent pouches.

Pleonectyptera. Clasper ovate, armature weak; uncus moderately clavate.

Phytometra. Apical portion of clasper slightly produced, armature weak; uncus falcate.

Alabama and *Salia.* Clasper slender; without armature; sacculus strong; apex produced beyong costal margin.

STUDIES OF CERTAIN PHASES OF THE BIOLOGY OF THE CHINCH BUG, BLISSUS LEUCOPTERUS (SAY), UNDER CONDITIONS OF CONSTANT TEMPERATURE AND CONSTANT RELATIVE HUMIDITY[1]

M. J. JANES

From the Department of Zoology and Entomology, Iowa State College

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The chinch bug, *Blissus leucopterus* (Say), is one of the most destructive insects to North American agriculture. A review of the literature shows that little detailed study has been made on certain phases of the biology of this insect. In the fall of 1933 studies were begun on the oviposition of the chinch bug. These were followed by experiments on starvation and drowning and later by experiments on the incubation of the egg.

Studies were made of the oviposition and longevity of 34 pairs of overwintered chinch bugs, 34 pairs of first generation and 13 pairs of second generation chinch bugs. The experiments were conducted under conditions of controlled temperature and relative humidity. Temperatures employed were 24.5° C., 29.5° C. and 34.5° C. Relative humidity was maintained within the range of 70 per cent to 100 per cent.

The insects were caged on young growing wheat shoots by means of test tubes, one male and one female in each cage. The eggs were laid in cotton batting around the base of the food plant. Egg counts were made regularly at 24-hour intervals. Each female was dissected at the time of her death and the eggs contained in the ovaries, visible with the aid of a binocular microcsope, were counted. The insects used in the experiments consisted entirely of material taken from the field. The overwintering adults were collected in favorable grassy hibernation quarters. First and second generation adults were obtained by selecting fifth instar nymphs from the field and then caging them in the laboratory until they reached the imaginal stage.

In every case, with respect to the various generations, the highest total individual egg-production occurred at 29.5° C. This was also true for the average total production. The greatest number of eggs laid by a female in one day occurred in every case at 34.5° C. Longevity decreased with each successive increase in temperature.

Overwintered females laid an average of 544 eggs. Although oviposition periods decreased with each successive increase in temperature, females at the higher temperatures laid a greater number of eggs per day. The average number laid by females at 24.5° C. was 532; at 29.5° C., 598; and at 34.5° C. the average was 502. The highest individual production was 1,091 eggs laid over a period of 72 days by a female kept at 29.5° C. The females contained but few eggs in their ovaries at the time of death. The preoviposition period following emergence from hibernation was low in most cases. The shortest period was 2 days and the longest 12

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days, occurring respectively at 34.5° C. and 29.5° C. The oviposition period varied from a minimum of 25 days at 34.5° C. to a maximum of 110 days at 24.5° C. Postoviposition periods usually did not extend beyond 1 or 2 days. Longevity of males exceeded that of females and in one case a male lived 138 days or over four months.

First generation females laid an average of 495 eggs. The average at 24.5° C. was 424, at 29.5° C. it was 548 and at 34.5° C. the average was 490. The highest individual record was 686 and the lowest 247. Preoviposition periods were slightly longer than those of the overwintered insects. Oviposition periods averaged 64 days at 24.5° C., 47 days at 29.5° C. and 32 days at 34.5° C. Most of the females died shortly after egg-laying ceased.

Egg-production by second generation chinch bugs was considerably reduced. The average total eggs per female was 352. One female kept at 29.5° C. produced 594 eggs over a period of 56 days. The average total number of eggs laid by females at this temperature was 475. At 24.5° C. the average was 225 and at 34.5° C. it was 306. Oviposition periods of females ranged from a minimum of 21 days at 34.5° C. to a maximum of 63 days at 24.5° C. Length of life varied from 12 days for a male at 29.5° C. to 86 days for two males, respectively, at 29.5° C. and 24.5° C. At 29.5° C. the average longevity of the females was 62 days and of males, 58 days. This was the only case encountered during the entire season in which the average life of the females exceeded that of the males.

Studies on the incubation of the chinch bug were conducted during the summer of 1935. Various conditions of constant temperature and constant relative humidity were employed. A total of 2,400 eggs was used, 100 at each combination of temperature and relative humidity. The temperatures employed were 19.5° C., 24.5° C., 29.5° C. and 34.5° C. The relative humidities were 20 per cent, 40 per cent, 60 per cent, 80 per cent and 100 per cent. In addition one lot of eggs was incubated on wet blotting paper kept saturated with distilled water. Readings were taken at 4-hour intervals.

Eggs hatched in approximately 30 days at 19.5° C., 15 days at 24.5° C., 10 days at 29.5° C. and 7 days at 34.5° C. Temperature seemed not to affect appreciably the percentage of hatch. In each case there was considerable spread in the time of hatching of a given lot of eggs subjected to the same conditions. Relative humidity although apparently influencing the length of the incubation period to a slight extent had its greatest effect on the percentage of hatch. The most favorable humidity at the higher temperatures was 80 per cent. Chinch bugs hatched after submergence in water for as long as 15 days. An increase in the incubation period occurred in fairly direct proportion to the period of submergence.

Several thousand chinch bugs representing the various instars were studied during the summer of 1934 to determine the ability of the chinch bug to withstand starvation and submergence in water. The insects remained alive and were able to resume normal activities after being deprived of food and water for periods varying from 8 hours to 13 days. High temperatures or low relative humidities were most destructive to the starving insects. Access to distilled water or to solutions of sucrose increased the longevity. Various combinations of constant temperature and humidity were maintained in this study. Chinch bugs were able to recover in some cases after submergence in water for as long at 48 hours.

SOME APPLICATIONS OF THERMIONIC VACUUM TUBES IN PHYSICAL CHEMISTRY[1]

MYRON TRUMAN KELLEY

From the Department of Chemistry, Iowa State College

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The purpose of this work was to improve the vacuum tube measuring devices now used in physical chemical measurement by making them suitable for operation from alternating current power lines and by making use of recently developed vacuum tubes. The work was limited to those instruments used in the measurement of galvanic potentials and the measurement of electrolytic conductance. It was the primary intent of the writer to develop simple devices suitable for use in the determination of end points of electrometric titrations, although some of the circuits are suitable for use as precision measuring devices.

Probably the most important feature of the circuits described is the use of neon glow lamps to reduce the effect of line voltage variations upon the accuracy of the measurements. These lamps are particularly efficient in removing the effect of the semi-periodic fluctuations which occur on heavily loaded a-c lines. The regulation characteristics of these neon glow lamps were measured, and a mathematical analysis of the parameters of the regulating circuits was made to find the most favorable values. This condition was found when the current limiting resistor for the lamp had the maximum permissible value for the load being applied.

Three circuits were developed for vacuum tube voltmeters to be used as continuous reading potential indicating devices in performing potentiometric titrations. The first is an a-c operated version of Goode's (1) simple vacuum tube voltmeter, the second a simplified bridge circuit similar to that of Garman and Rdoz (2), and the third a cascade resistance-coupled circuit. The second and third circuits are most satisfactory because they show better stability with respect to line voltage variation. The third circuit is most satisfactory in general because of its greater sensitivity and its simplicity of operation. This circuit is also desirable in that it can be used to determine the end-points of titrations using the glass electrode, although it is not suitable for exact pH determinations. The scales of all these voltmeters are nearly linear with respect to applied voltage. The sensitivities of the instruments are: for the first, 0.65 milliamperes per volt; for the second, 0.4 milliamperes per volt; and for the third, 1.1 milliamperes per volt.

Neon glow lamp voltage regulation was applied to the "sectrometer" of G. Frederick Smith (3) to eliminate "wandering" of the shadow and to simplify the circuit. Two circuits were developed, one having a sensitivity of 0.1 volt for 90° throw of the cathode ray shadow, and the other 0.3 volt for the same movement.

This regulation principle has been applied to the ballistic vacuum tube galvanometer circuit developed by Hemingway (4) so that it may be

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operated entirely from the a-c line. When used with any standard potentiometer set-up (properly shielded and grounded), this instrument can be used to determine potentials of high resistance electrodes (such as glass electrodes) with an accuracy of one millivolt. This accuracy can be increased by the addition of a carefully shielded resistance capacity coupled amplifying stage.

For the measurement of electrolytic conductance two thermionic bridge balance indicators have been employed. The first of these makes use of an RCA type 6E5 cathode ray tube in a circuit similar to that of Breazeale (5). However, the method of balancing has been changed to make the operation of the instrument more satisfactory when used with low frequency a-c bridge current supplies. This improvement was accomplished by making the first tube act as a plate-rectifying detector rather than as an amplifier. This bridge balance indicator is almost a duplicate of the modified "sectometer" circuit previously described, and the instrument can be used for both purposes by providing the proper switching arrangement.

The second bridge balance indicator is actually a cathode ray oscilloscope using the recently developed RCA 913 cathode ray tube. This method of bridge balancing allows an analysis of the bridge output near the balance point into its resistance component and the inductance- capacity component, and thus allows one to judge whether the latter effect is sufficiently large to justify applying corrective measures. The sensitivities of the two circuits as described are about the same, that is, about 0.1 per cent at a resistance value of 100 ohms, or about 0.5 per cent at 50,000 ohms. These sensitivities are sufficient for many routine determinations of conductivity in the physical chemistry laboratory.

A direct reading conductance meter has been described for use in determining the end-points of conductometric titrations. The scale of the instrument is nearly linear, and its sensitivity can be changed over a wide range. The accuracy of the instrument depends largely upon the constancy of the a-c source which is available. When a fairly constant source can be obtained, the instrument is very satisfactory for determining the end-points of a large variety of conductometric titrations.

The last part of the work deals with a-c sources for conductance measurement. An electron-coupled oscillator which has good wave form and a fairly high stability toward output load has been described. When designed to have the proper frequency, this oscillator is quite satisfactory for either auditory bridge balancing or for use with the visual cathode ray instruments which have been described. A method of stabilizing the output of this oscillator (using neon glow lamps) has been developed so that this device makes an excellent source for use with the direct reading conductance meter referred to above.

A series of a-c operated vacuum tube instruments for use in the measurement of galvanic potentials and electrolytic conductance have been developed and shown to be practical. These instruments are sturdy, compact units which can be applied in making routine determinations or in carrying out electrometric titrations. The cost of such devices is quite low because of the mass production of the component parts for use in the radio industry.

It is possible that neon glow lamp voltage regulation, which has been applied extensively in this research, may be applicable to other vacuum tube measuring devices with a resultant improvement in the characteristics of these instruments.

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ADDITION OF ORGANOMETALLIC COMPOUNDS TO CONJUGATED SYSTEMS[1]

Robert H. Kirby

From the Department of Chemistry, Iowa State College

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A study was made of the various factors influential in determining the course of the reactions of organometallic compounds with compounds having conjugated systems. Generalizations, helpful in predicting the products of untried reactions, were formulated and the importance of the relative reactivity of the organometallic compounds was emphasized.

A survey of the literature indicated that the groups in conjunction with the conjugated system play a definite part in determining the type of addition. The reactions of α-ethylenic ketones give saturated ketones (the 1,4-addition product) and

$$\begin{array}{lll} & \nearrow & \mathrm{RR''CHCH{=}\underset{\underset{\displaystyle OM}{|}}{C}R'} \xrightarrow{\mathrm{HOH}} \mathrm{RR''CHCH_2\underset{\underset{\displaystyle O}{\|}}{C}R'} \\ \mathrm{RCH{=}CH\underset{\underset{\displaystyle O}{\|}}{C}R'} \xrightarrow{\mathrm{R''M}} & & \\ & \searrow & \mathrm{RCH{=}CH\underset{\underset{\displaystyle OM}{|}}{C}R'R''} \xrightarrow{\mathrm{HOH}} \mathrm{RCH{=}CH\underset{\underset{\displaystyle OH}{|}}{C}R'R''} \end{array}$$

unsaturated carbinols (the 1,2-addition product). Four simple rules were found applicable to such reactions. First, the influence of R′ upon the type of addition is similar to the effect of R′ upon the rate of reaction of the common carbonyl reagents with the simple ketones, CH_3COR' [2]. Thus, the amount of 1,2-addition decreases as R′ changes in the order: CH_3, C_2H_5, $(CH_3)_3C$, C_6H_5, 2,4,6-$(CH_3)_3C_6H_2$. When R′ is a hydrogen atom, it has been found that, with the exceptions of the *tert.*-butyl and *tert.*-amyl magnesium compounds, Grignard reagents give largely 1,2-addition[3]. Second, a group or groups on the β-carbon atom tend to decrease the amount of 1,4-addition, a phenyl group having greater influence than methyl and two groups having more effect than one. Third, if R′ is an aromatic radical, the 1,2-directing influence of monosubstitution on the β-carbon atom is completely overcome and 1,4-addition occurs. Fourth, substitution on the α-carbon atom appears to favor 1,4-addition[4]. These rules also apply in a general way to the reactions of alicylic α-unsturated ketones. The reactions of a variety of compounds having conjugated systems composed of the elements, carbon, oxygen, nitrogen and sulfur,

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[1]Original thesis submitted June, 1937. Doctoral thesis No. 429.

[2]Kohler, *Am. Chem. J.*, 38: 511 (1907).

[3]Stevens, *J. Am. Chem. Soc.*, 57: 1112 (1935).

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were summarized, but the application of the above-mentioned rules was limited by insufficient data.

Temperature and solvent have been found to have little effect upon the amounts of 1,2- and 1,4-addition[2].

By comparing the reactions of a given congugated system and various Grignard reagents, the tendency of these organometallic compounds to give 1,2-addition was found to increase in the order: *tert.*-alkyl, alkyl, aryl, methyl. Exceptions to this series were pointed out. In a general way the series follows the order of reactivity of Grignard reagents found by Gilman and co-workers, the less reactive organometallic compounds favoring 1,4-addition.

The halogen atom, percentages of R_2Mg and RMgX, and steric hindrance of Grignard reagents were found inadequate in explaining the reactions of conjugated systems.

While many conjugated systems give the same type of addition with organometallic compounds having the same R-group and different metallic components, two reactions have been reported in which a variation in the metal resulted in different products. Benzophenone-anil[5] and chalcone[6] gave 1,4-addition with phenylmagnesium bromide and 1,2-addition with phenyllithium. This thesis extends such reactions to include the phenyl derivatives of Zn, Al, Mn, Be, Ca, Na and K. The reactions were carried out in the conventional manner and the yields are given in parenthesis.

Chalcone gave β,β-diphenylpropriophenone with diphenylzinc (91 per cent), triphenylaluminum (94 per cent), phenylmanganous iodide (77 per cent), and diphenylberyllium (90 per cent). Chalcone with phenyllithium and phenylsodium gave β,β-diphenylpropiophenone (13 and 3.5 per cent, respectively) and diphenylstyrylcarbinol (69 and 39 per cent, respectively. Chalcone gave diphenylstyrylcarbinol with phenylcalcium iodide (45 per cent) and phenylpotassium (52 per cent).

4-Dimethylaminochalcone gave β-(*p*-dimethylaminophenyl)-β-phenylpropiophenone with diphenylberyllium (71 per cent) and phenylmagnesium bromide (52 per cent). Phenyllthium gave the same ketone (14 per cent) and also *p*-dimethylaminostyryldiphenylcarbinol (76 per cent) (m.p 117°. This carbinol was isolated in a 64 per cent yield form the reaction of 4-dimethylaminochalcone and phenylcalcium iodide.

The product of the reaction of 4-dimethylaminochalcone and phenylmagnesium bromide was originally reported as *p*-dimethylaminostyryldiphenylcarbinol (m.p. 100°)[7]. This reaction was reinvestigated and the substance melting at 100° was characterized as β-(*p*-dimethylaminophenyl)-β-phenylpropiophenone. An analysis by the Zerewitinoff method failed to indicate an active hydrogen and the compound was synthesized from chalcone and *p*-dimethylaminophenylmagnesium iodide in a 71 per cent yield. Chalcone and *p*-dimethylaminophenyllithium gave the same ketone (12 per cent) and also *p*-dimethylaminophenylphenylstyrylcarbinol (76 per cent) (m.p. 131°).

The preparation of *p*-dimethylaminophenylcalcium iodide from *p*-iododimethylthylaniline and calcium was found unsatisfactory.

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[5]Gilman and co-workers, *J. Am. Chem. Soc.*, **51**:2252 (1929); *ibid.*, **55**:1265 (1933).
[6]Kohler, *Am. Chem. J.*, **31**:642 (1904; Lüttringhaus, *Ber.*, **67*B***:1602 (1934).
[7]MacLean and Widdows, *J. Chem. Soc.*, **105**:2169 (1914).

Diphenylberyllium was prepared from beryllium chloride and phenyllithium. The product of the reaction with chalcone, β,β-diphenylpropiophenone, established that the phenylberyllium compound was in hand, for phenyllithium would have given largely diphenylstyrylcarbinol. Chalcone was likewise used as a reagent for the identification of *p*-dimethylaminophenylmagnesium iodide (prepared from magnesium iodide and *p*-dimethylaminophenyllithium) and phenylmanganous iodide (prepared from manganous iodide and phenyllithium).

Benzophenone-anil gave triphenylmethylaniline with phenylsodium (1.5 per cent) and phenylpotassium (73 per cent).

These data divide the phenylmetallic compounds into groups, the derivatives of Zn, Al, Mn, Be and Mg which gave 1,4-addition, the derivatives of Li and Na which gave largely 1,2-, but some 1,4-addition and the derivatives of Ca and K which gave only 1,2-addition. Gilman and co-workers have found that the relative reactivity of the organometallic compounds which gave largely or exclusively 1,2-addition is definitely greater than that of the compounds which gave 1,4-addition, and it appears that in general high reactivity favors 1,2-addition.

THE EFFECT OF CERTAIN STIMULANTS UPON THE GROWTH OF YEAST[1]

James B. Lesh

From the Department of Chemistry, Iowa State College

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The first definite indication of the existence of a yeast growth stimulant appeared when Wildiers (1901) reported that he was unable to grow yeast in a solution of sugar and salts without the addition of a small amount of wort, yeast water, or beef extract. The unknown substance, or substances, to which the name "bios" was given until its identity could be established, was organic in nature. Attempts to replace the material with compounds of known composition were unsuccessful.

Progress in the isolation and identification of bios has been hindered by the lack of a standardized technique for the determination of stimulative activity. The use of different strains of yeast, and different media, has led in many cases to contradictions in the published results. It has been generally accepted that bios is of multiple nature. Lucas (1924) separated the stimulant from malt sprouts into two fractions, Bios I and Bios II, which were inactive alone, but gave large crops when combined. Bios I was identified as i-inositol by Eastcott (1928), and its complementary action has been confirmed by several investigators. The extent of its activity has been questioned, however, by others who were unable to obtain similar results. Fractionation of the growth stimulant from various other sources has been accomplished. The stimulant produced during the growth of molds on synthetic media was studied by Schopmeyer (1931), who was unable to obtain from it the Bios I and Bios II of Lucas. The purpose of this investigation was to compare the Bios II fractions obtained from molds and from malt sprouts, and to determine the effect of inositol and Bios II upon the growth of various strains of yeast in several media.

The growth stimulant produced by *Aspergillus niger* from a synthetic medium was fractionated into Bios I and Bios II by the method of Lucas. The extract of malt sprouts was treated similarly. The Bios I fractions were discarded because the i-inositol was available. The Bios II fractions were purified, and their effects upon yeast growth, with and without inositol, were determind. The yeast employed was a strain of *Saccharomyces cerevisiae* which had been isolated from a cake of Fleischmann yeast. The medium contained ammonium chloride, dipotassium phosphate, and sucrose. Determination of growth was made after twenty-four hour incubation of the cultures at 30° C.

The mold-produced stimulant was found to be similar to that obtained from malt sprouts. In both cases, however, the Bios II fractions were highly stimulative in the absence of inositol. The addition of inosital had very little effect on the growth of the yeast.

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The addition of magnesium sulfate to the medium was found to increase considerably the activity of the Bios II, and some complementary action of inositol was observed in the higher concentrations of the salt. The same effect was obtained by the use of magnesium chloride or magnesium nitrate only when the medium also contained a sulfate. The salts of calcium did not replace those of magnesium.

The effect of inositol, magnesium sulfate, and Bios II upon the growth of twenty strains of *Saccharomyces cerevisiae* was then determined. It was shown that in no case was the growth markedly affected by the addition of inositol, magnesium sulfate, or a combintaion of the two. All of the strains gave greater growth in the presence of inositol and Bios II when magnesium sulfate was added. The strains differed in several other respects which permitted their division into the following four convenient groups:

Group I. Bios II alone does not give increased growth.

Group II. Inositol does not give increased growth in the presence of Bios II when magnesium sulfate is absent. Six strains were placed in this group.

Group III. Magnesium sulfate does not give increased growth in the presence of Bios II. For the six strains in the above group, the growth was actually decreased by the addition of magnesium sulfate in the absence of inositol.

Group IV. This group includes those strains which show increased growth under the conditions given for Groups I, II, and III. The group includes five strains.

The differences in growth of the various strains of yeast show some reasons for discrepancies in the published results of bios studies where different strains of yeast and different media have been employed. The yeast used by Schopmeyer belongs in group II. The mold-produced stimulant was found to be different only because the strain of yeast shows no inositol effect in the medium then employed .

A study was made of the effect of inositol upon the clumping of cells. It was found that inositol reduces the tendency of the cells to form clumps. The clumping of cells was most pronounced with the strains of Group III.

Comparison was made of the growth of various strains of yeast in several synthetic media which have been used in bios studies. No single medium was found which gave the best results with all of the strains studied.

The addition of inositol to strains of Group III in media containing Bios II and magnesium sulfate results in very marked stimulation. The properties of Bios I and Bios II as described by Lucas (1924) are confirmed by the use of strains of Group III.

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CATALYTIC HYDROGENATION OF FURFURAL IN THE LIQUID PHASE AT VARIOUS TEMPERATURES AND PRESSURES[1]

RALPH E. MENZEL

From the Department of Chemistry, Iowa State College

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Hydrogenation of both elements and compounds, practiced on the colossal scale that it is today, has evolved from the admirable investigations of Sabatier and his associates, whose studies on the catalytic activity of finely divided metals were first reported in 1897 and for which, with later investigations, he was awarded the Nobel prize in chemistry in 1912.

Liquid phase hydrogenation of furfural has certain advantages over the gas phase. The by-products are fewer, especially when the proper conditions are utilized in the process, and when consideration is given to the sensitivness of furfural and furfuryl alcohol to long time contact with heat. This makes separations unnecessary or much easier.

Copper-chromium oxide catalysts seem to be better for the production of furfuryl alcohol and pentanediol-1,2 and 1,5 from furfural than the nickel catalysts (1, 2, 3). Nickel catalysts appear to produce tetrahydrofurfuryl alcohol from furfural more readily than the copper-chromium oxide catalysts (4, 5). The copper catalysts are also less subject to poisoning and do not need reactivation which is necessary with the platinum group catalysts.

EXPERIMENTAL

The rate of hydrogenation of furfural in the liquid phase was investigated in a rocking type, copper lined, electrically heated autoclave of approximately 3 feet in length and an inside diameter of about 3 inches and having a capacity of nearly 3.85 liters. Eight and six-tenths grams of catalyst in the 250 ml. of furfural was used in each experiment except where otherwise indicated. Temperatures up to 237°C. were used with the following catalysts at the pressures mentioned:

(A) Copper-chromium oxide catalyst prepared by precipitating copper, chromium, and barium from the nitrates with ammonium carbonate and used at initial pressures of 200 to 1,800 pounds.

(B) Copper-chromium oxide catalyst prepared by mixing CrO_3 solution and $CuCO_3$ powder, adding NH_4OH and drying the mixture. This was used alone (8.6 grams) and with 5 grams of CaO, $Ca(OH)_2$ (10 grams), BaO, $Ba(OH)_2$ and $Ba(OH)_2 \cdot 8H_2O$ at an initial pressure of 1,400 pounds.

(C) Cuprous oxide prepared by the use of dextrose on a solution of copper nitrate, and modifications of this by adding 0.089 moles of CaO, $Ca(OH)_2$, BaO, $Ba(OH)_2$, $Ba(OH)_2 \cdot 8H_2O$, and MgO at initial pressure of 1,400 pounds.

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(D) Initial pressure of 1,400 pounds and:

(1) Three different copper-chromium oxide catalysts prepared by precipitating copper, chromium and barium from the nitrates with ammonium carbonate solution.

(2) Calcium oxide (5 grams) mixed with copper-chromium oxide (8.6 grams), prepared from CrO_3 solution and $CuCO_3$ powder, adding NH_4OH and drying the mixture.

(3) Cuprous oxide (from dextrose) and $Ca(OH)_2$ (coprecipitated).

(4) Calcium hydroxide (5 grams) mixed with copper-chromium oxide catalyst (8.6 grams) prepared by precipitating copper and chromium from a solution of copper sulfate and sodium dichromate, with ammonium hydroxide.

(5) Cuprous oxide (from dextrose) (8.6 grams) and calcium oxide (5 grams) mixed.

(E) Cuprous oxide (from dextrose) (8.6 grams) mixed with calcium oxide (5 grams) at initial pressures of 400, 600, 1,000, 1,200, 1,400, and 1,900 pounds.

(F) Copper-chromium oxide catalyst (Adkins 30 R.A.C.) (6) precipitated from the nitrates with ammonium carbonate compared to Cu_2O (from dextrose) (8.6 grams) and CaO (5 grams) at initial pressures of 600, 1,400, and 1,900 pounds.

(G) Cu_2H_2, Cu (powder), CuO, Cu_2O (Baker and Adamson), Cu_2O (prepared by reduction with SO_2) . Cu-Ur oxide, Cr_2O_3, NiO (green, Sargent and Co.) at 1,400 pounds initial pressure.

The autoclave was allowed to heat up as rapidly as possible by using the 220 volt current until the temperature of the liquid reached 237°C. and then allowed to cool while the reaction continued. The time required for the liquid in the autoclave to reach 237°C. was about 100 minutes. The rate of reaction with hydrogen was followed by taking the temperature and pressure readings every five minutes during the heating and cooling period. The pressures were calculated to 0°C., and from these pressures at 0°C. the amount of hydrogen which reacted was calculated for each five minute interval during the hydrogenations.

CONCLUSIONS

1. For any one catalyst the temperature at which hydrogenation starts is independent of the initial pressure of hydrogen between 200 and 1,800 pounds.

2. The temperature at which hydrogenation starts is different for different catalysts when using the same pressures.

3. Change of pressure has more effect on the rate of hydrogenation of furfural, in the presence of Cu-Cr oxide catalyst, at initial pressures of 200 to 600 pounds, than from 600 to 1,800 pounds.

4. In the hydrogenation of furfural at an initial pressure of 1,400 pounds, CaO, $Ca(OH)_2$, and $Ba(OH)_2$ increase the rate (in the order mentioned) of action of Cu-Cr oxide, but CaO decidedly more than the others. $Ba(OH)_2 \cdot 8H_2O$ is much more detrimental to the catalytic action of Cu-Cr oxide than BaO.

5. In the hydrogenation of furfural at an initial pressure of 1,400 pounds, CaO, $Ba(OH)_2$, $Ca(OH)_2$, and MgO increase the rate (in the order mentioned) of action of Cu_2O, but CaO decidedly more than the others. The total amount of hydrogen reacting is also increased. BaO is much more detrimental to the catalytic activity of Cu_2O than $Ba(OH)_2 \cdot 8H_2O$.

6. Change of pressure has more effect on the rate of hydrogenation of furfural in the presence of Cu_2O with CaO catalyst, at initial pressures of 400 to 1,000 pounds than from 1,000 to 1,900 pounds.

7. The only advantage of using more than 1,000 pounds of initial pressure in the hydrogenation of furfural to furfuryl alcohol is a slight increase in the rate of reaction.

8. The greatest drop in pressure (calculated to 0°C.) during any five minute interval was 170 pounds and was produced by Cu_2O with CaO catalyst at an initial pressure of 1,400 pounds.

9. The greatest total amount of hydrogen to react (5.27 moles) was produced by Cu-Cr oxide catalyst (Adkins 30 R.A.C.) (6) when using 1,900 pounds initial pressure.

10. Copper oxide-calcium oxide catalyst caused 2.25 moles of hydrogen to react in the least number of minutes (12.5) when using an initial pressure of 1,900 pounds.

11. The Cu_2O, CaO mixture is very similar to Cu-Cr oxide catalyst (Adkins 30 R.A.C.) (6) in its rate of action, and the Cu_2O, CaO catalyst is much easier and simpler to prepare.

12. Cuprous oxide prepared by reduction with SO_2, and Cu_2O (Baker and Adamson) were found to be inactive for the hydrogenation of furfural but Cu_2O prepared by the use of dextrose proved to have high activity. The method of preparing the Cu_2O catalyst is very vital to its activity.

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RELATIVE REACTIVITIES OF SOME ORGANOMETALLIC COMPOUNDS[1]

JOSEPH F. NELSON

From the Department of Chemistry, Iowa State College

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Hydrogen attached to oxygen, sulphur, selenium, tellurium, nitrogen, phosphorus, the triple-bond carbon in the acetylenes, and to certain other types of carbon atoms, such as the *alpha* carbon atoms in the furans, the fourth and sixth carbons in the dibenzofurans, and the methylene carbon in indene and similar compounds is known as active hydrogen. Tschugaeff[2] and Zerewitinoff[3] have developed a method for the quantitative estimation of active hydrogen attached to oxygen, sulphur, selenium, nitrogen, and the triple-bond carbon. The compound containing the active hydrogen is treated with methylmagnesium iodide and the amount of active hydrogen present is calculated from the quantity of methane evolved.

The present investigation deals with hydrogen attached to oxygen, sulphur, selenium, nitrogen, and the triple-bond carbon. Due to the variation in reactivity of such hydrogen atoms, it has been found possible to differentiate them by the use of various aliphatic organometallic compounds. The apparatus used for the purpose is that devised by Zerewitinoff[3].

A twenty per cent by volume solution of triethylaluminum *n*-butyl-etherate (boiling point 140-141°/15 mm., $D^{27}_4 = 0.8182$) in *n*-butyl ether reacts with alcohols, phenols, enols, oximes, carboxylic acids, and thiols; when heated at 100° for one hour. It likewise reacts with hydrogen attached to nitrogen in the amines, the amides, the ketimines, etc. Reaction with some secondary amines is very slow; consequently, longer periods of heating are required. It fails to react or shows very little reaction with the acetylenes. Thus, this reagent will differentiate hydrogen attached to oxygen, sulphur, or nitrogen from that attached to the triple-bond carbon in the acetylenes.

A twelve per cent by volume solution of diethylzinc in *n*-butyl ether reacts with all the above mentioned types of active hydrogen. Secondary amines show little or no reaction at the end of a ten-minute period at 100°, whereas primary amines show considerable reaction in this time interval. Consequently, the two classes may be differentiated with this reagent.

A solution of diethylcadmium in *n*-butyl ether (15 to 17 grams per 100 cc. of solution) reacts with hydrogen attached to oxygen, sulphur, and to nitrogen which is attached to a negative element or to a carbon atom that is attached to a negative element, when heated at 100° for twenty minutes. Thus, these types of active hydrogen may be differentiated from

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[1] Original thesis submitted June, 1937. Doctoral thesis number 433.

[2] Tschugaeff, *Ber.* 35, 3912 (1902); *ibid.*, 42, 49 (1909).

[3] Zeriwitinoff ,*Ber.*, 40, 2023 (1907); *ibid.*, 41, 2233 (1908); Z. *anal. Chem.*, 59, 680 (1911).

hydrogen attached to nitrogen in the remaining amines and to carbon in the acetylenes. The reaction with phenylacetylene is slow.

A twenty-five per cent by volume solution of tri-*n*-propylboron in xylene or *n*-butyl ether reacts with the carboxylic acids, the oximes, the amines, and the thiols, but not with the alcohols, the amines, the acetylenes, and apparently not with the selenols. Reaction appears to take place with most enols and the more highly negatively substituted phenols, but not with the simpler phenols. The reaction with the phenols and enols appears to be a function of their acidities. This differentiation depends on heating at 100° for 65 minutes. The evolved gas must be measured while the Zerewitinoff bulb is immersed in a water-bath at 100°, due to the solubility of propane in the solvents used.

Tetraethyltin in *n*-butyl ether shows very little or no reaction with all of the above mentioned types of active hydrogen, when heated at 100° from one to three hours. Even selenophenol, which readily cleaves the organolead compounds, shows little or no reaction. Likewise, selenophenol does not react wtih tetraethylsilicon.

Diethylmercury in *n*-butyl ether does not react with the acetylenes, but it does with the selenols and the thiols. The aliphatic thiols react quite slowly, and the reaction is quite incomplete.

Hydrogen attached to sulphur and selenium may be differentiated from all other types of active hydrogen (strong carboxylic acids excepted) by the use of tetraethyllead or triethylbismuth[4], since these two organometallic compounds react only with the -SH and -SeH groups, and the very strong carboxylic acids.

Unfortunately, the reaction between organometallic compounds of lesser reactivity than the Grignard reagent, and active hydrogen compounds is not quantitative. Only about six- to eight-tenths of the active hydrogen is evolved as ethane or propane. These low results are no doubt in part due to the solubility of these gases in the solvents used. Haurowitz[5] in his studies with diethylzinc found similar low results.

It is well-known that nitro, nitroso, and azo groups interfere with the Zerewitinoff determination[6]. They, likewise, interfere in the reaction between most of the lesser reactive organometallic compounds and active hydrogen-containing compounds. The nitro group was observed to interfere in the determinations with the aluminum, cadmium, zinc, boron, and lead compounds; the nitroso group with the boron compounds; and the azo group with aluminum, cadmium, boron, and zinc compounds. The nitro group does not interfere in the determinations with triethylbismuth; and the azo group does not interfere in the case of either triethylbismuth or tetraethyllead. Active hydrogen determinations do not appear to be affected by steric hindrance.

The amount of active hydrogen has been found to vary with the choice of solvent. Pyridine may not be used as a solvent in the procedure outlined herein for the differentiation of active hydrogen.

From the standpoint of the organometallic compounds used, the results may be interpreted so as to arrange these compounds in the order of

[4] Gilman and Nelson, *J. Am. Chem. Soc.*, 59, 935 (1937).

[5] Haurowitz, *Mikrochemie*, 6, 88 (1929).

[6] Gilman and co-workers, *J. Am. Chem. Soc.*, 49, 2815 (1927); *ibid.*, 50, 867 (1928); *ibid*, 52, 405 (1930); *Bull. soc. chim.*, 4 45, 1132 (1929).

their reactivity toward active hydrogen. Diethylzinc reacts with all the active hydrogen types investigated; triethylaluminum fails to react with the acetylenes and most amines; and tri-*n*-propylboron fails to react in addition to these with many compounds containing the -OH group. Tetra-atheyllead reacts only with the -SH and -SeH groups and a few of the strong carboxylic acids; diethylmercury shows less reactions with the -SH group and according to the results obtained by Catlin[7] the bismuth compounds are still less reactive. As mentioned before, tetraethyltin shows little or no reaction with the active hydrogen types investigated, and tetraethylsilicon is still less reactive.

Thus, the order of reactivity toward active hydrogen is

$$\mathrm{Zn} > \mathrm{Al} > \mathrm{Cd} > \mathrm{B} > \mathrm{Pb} > \mathrm{Hg} > \mathrm{Bi} > \mathrm{Sn} > \mathrm{Si}.$$

Inclusion of published results of active hydrogen reactions with the more reactive organometallic compounds extends the series to

$$\mathrm{K} > \mathrm{Na} > \mathrm{Li} > \mathrm{Ca} > \mathrm{Mg} >?\ \mathrm{Zn} > \mathrm{Al} > \mathrm{Cd} > \mathrm{B} > \mathrm{Pb} > \mathrm{Hg} > \mathrm{Bi} > \mathrm{Sn} > \mathrm{Si}$$

The reaction of the lead, mercury, and bismuth compounds with the -SH and -SeH groups in anomalous, and of the following order:

$$\mathrm{Bi} > \mathrm{Pb} > \mathrm{Hg}.$$

[7] Catlin, "Some Physico-Chemical Studies of Organometallic and Furan Compounds," Thesis, Library, Iowa State College, 1934, p. 67.

ANALGESICS FROM DIBENZOFURANS[1]

PAUL T. PARKER

From the Department of Chemistry, Iowa State College

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Contained within the chemical structure of morphine are the skeletal structures of phenanthrene and dibenzofuran. Recent studies have shown that many of the simpler phenanthrene derivatives possess significant analgesic activity. Also, a comparison of the analgesic action of a number of representative compounds of phenanthrene and dibenzofuran has indicated[2] that the derivatives of dibenzofuran are equally as effective as the corresponding derivatives of phenanthrene in this respect.

In connection with the studies which are being carried on in this laboratory on the chemistry and orientation of dibenzofuran, a series of derivatives has been synthesized and examined for analgesic activity. The purpose of the work reported in this thesis has been to extend and amplify this series of dibenzofuran compounds with the specific intent of preparing compounds holding promise of being analgesically active.

By heating 2-acetyldibenzofuran with ammonium formate a small yield of 2-α-aminoethyldibenzofuran hydrochloride (m. 222-223°) was obtained. Methyl-2-dibenzofurylcarbinol[3] was prepared from 2-dibenzofurylmagnesium bromide and acetaldehyde. The carbinol was converted to the bromide and treated with diethylamine to obtain the 2-α-diethylaminoethyldibenzofuran. This compound was an oil which, because of the extreme hygroscopicity of its salts, was characterized as the picrate (m. 173-174°).

Carbonation of the Grignard reagent from 1-bromo-4-methoxydibenzofuran yielded 4-methoxy-1-dibenzofurancarboxylic acid (m. 279-280°). The same Grignard reagent and ethylene oxide furnished 1-β-hydroxyethyl-4-methoxydibenzofuran (m. 96-96.5°), which was converted to the bromide and treated with diethylamine. The 1-β-diethylaminoethyl-4-methoxydibenzofuran thus obtained was an oil and was isolated and purified as the hydrochloride (m. 187°; decompn.).

Treatment of 2-nitro-3-acetaminodibenzofuran with stannous chloride and hydrochloric acid in hot acetic acid solution gave 2-methyl-1-benzofuro-[2,3-f]-benzimidazole (m. 270°). The hydrochloride does not melt up to 335°.

The acetylation of 4- methoxydibenzofuran gave 1-acetyl-4-methoxydibenzofuran (m. 134-134.5°). The oxime melted at 176-177.5°. The position of the acetyl group was established by oxidation to the corresponding acid which was identical with the 4-methoxy-1-dibenzofurancarboxylic acid obtained from 1-bromo-4-methoxydibenzofuran. A. Beckmann rearrangement of the oxime yielded 1-acetamino-4-methoxydibenzofuran (m. 222-223°), the nitration of which gave 1-acetamino-2-nitro-4-methoxydi-

[1] Original thesis submitted June, 1937. Doctoral thesis number 434.

[2] Eddy, *J. Pharmacol.*, 58, 159 (1936).

[3] Mosettig and Robinson, *J. Am. Chem. Soc.*, 57, 2186 (1935).

benzofuran (m. 244°). The position of the nitro group in this compound was shown by converting it to 2-methyl-5-methoxy-1-benzofuro-[3,2-e]-benzimidazole (m. 222-222.5°; hydrochloride, m. 306-307°; decompn.) by means of a stannous chloride and hydrochloric acid reduction in hot acetic acid solution. Hydrolysis of the 1-acetamino-2-nitro-4-methoxydibenzofuran gave the 1-amino-2-nitro-4-methoxydibenzofuran (m. 206-207°) which was diazotized and boiled with dilute alcohol to obtain the 2-nitro-4-methoxydibenzofuran (m. 185-186°). Catalytic reduction of this compound gave 2-amino-4-methoxydibenzofuran (m. 127-127.5°).

Nitrourea and 3-aminodibenzofuran in alcohol solution yielded 3-dibenzofurylurea, which melted at 222-223° on a nickel block with immediate resolidification. The compound is relatively insoluble in the usual organic solvents. The condensation of 2-acetyldibenzofuran with trioxymethylene and dimethylamine hydrochloride yielded 2-β-dimethylaminopropionyldibenzofuran (m. 88-89°; hydrocloride, m. 195-196°). From the reaction between 4-dibenzofuryl-lithium and isoquinoline by the method of Ziegler and Zeiser[4] was obtained 1-(4-dibenzofuryl)-isoquinoline (m. 137-138°). The hydrochloride of this compound was not stable in aqueous solution.

The thiocyanogenation of 3-aminodibenzofuran by the method of Kaufmann and Oehring[5] gave a thiocyanate melting at 175° with immediate resolidification. This compound was considered to be 3-amino-2-dibenzofurylthicyanate, since thiocyanogenation is analogous to halogenation, which has been shown to take place in the 2-positions in this case.[6] Treatment with alcoholic hydrochloric acid rearranged this derivative to 2-aminobenzofuro-[2,3-f]-benzothiazole (m. 268-269°). The hydrochloride decomposes above 300°.

A Friedel-Crafts reaction of succinic anhydride with dibenzofuran yielded the previously reported β-2-dibenzofuroylpropionic acid[6]. By a Clemmensen reduction of this compound was obtained γ-2-dibenzofurylbutyric acid[7]. Cyclization of this acid was accomplished with 88 per cent sulfuric acid, the product being a derivative of either β- or γ-brazan, 1-keto-1,2,3,4-tetrahydro-β(or γ)-brazan (m. 137°). The oxime melted at 212-213°. Reduction of the oxime with sodium amalgam gave 1-amino-1,2,3,4-tetrahydro-β(or γ)-brazan which was isolated as the hydrochloride (m.266-267°). Condensation of the keto-tetrahydrobrazan with trioxymethylene and dimethylamine hydrochloride yielded 1-keto-2-dimethylaminoethyl-1,2,3,4-tetra-hydro-β (or γ)-brazan hydrochloride (m. 185-186°).

Mr. J. C. Bailie has shown in unpublished work that 3-nitrodibenzofuran can be successfully acetylated in a Friedel-Crafts reaction to give 2-acetyl-7-ntirodibenzofuran (m. 212-213°). From the catalytic reduction of this compound was obtained 2-acetyl-7-aminodibenzofuran (m. 158-159°) which was acetylated to the 2-acetyl-7-acetaminodibenzofuran (m. 203°). The oxime of this compound also melted at 203°, but a mixed melting point was strongly depressed. Nitration of the 2-acetyl-7-acetaminodibenzofuran gave 2-acetyl-7-acetamino-8-nitrodibenzofuran (m. 270-

[4] Ziegler and Zeiser, *Ann.*, 485, 174 (1931).

[5] Kaufmann and Oehring, *Ber.*, 59, 187 (1926).

[6] Gilman, Brown, Bywater and Kirkpatrick, *J. Am. Chem. Soc.*, 56, 2473 (1934).

[7] Mayer and Krieger, *Ber.*, 55, 1659 (1922).

271°), which was converted to 2-methyl-8-acetyl-1-benzofuro-[2, 3-f]-benzimidazole (m. 298°) by catalytically reducing the nitro group and refluxing the product with glacial acetic acid. The hydrochloride decomposes at approximately 325°.

Diazomethyl 4-dibenzofuryl ketone, prepared from 4-dibenzofurancarboxylic acid chloride and diazomethane, was rearranged to 4-dibenzofurylacetamide (m. 211-212°) by the general method of Arndt and Eistert[8]. Hydrolysis of the amide yielded 4-dibenzofurylacetic acid (m. 213.5-214.5°). The 4-dibenzofurylacetyl chloride, obtained by treating the acid with thionyl chloride, was reacted with 3,4-dimethoxy-α-(dibenzofurylacetamino)-acetophenone (m. 186-187°). Similarly, from the reaction between 4-dibenzofurancarboxylic acid chloride and 3,4-dimethoxy-α-aminoacetophenone there was obtained 3,4-dimethoxy-α-(dibenzofuroylamino-acetophenone (m. 178-179°).

The nitration of 1-bromo-4-methoxydibenzofuran gave a mononitro derivative (m. 160-161°), which was shown to be 1-bromo-3-nitro-4-methoxydibenzofuran by a catalytic reduction and dehalogenation to the known as 3-amino-4-methoxydibenzofuran (m. 75-76°)[9]. The 1-bromo-3-amino-4-methoxydibenzofuran (m. 135-136°) was obtained by reduction of the nitro compound with stannous chloride and hydrochloric acid. Acetylation gave the 1-bromo-3-acetamino-4-methozydibenzofuran melting at 178-179°.

[8] Arndt and Eistert, *Ber.*, 68, 200 (1935).

[9] Unpublished work by Mr. A. L. Jacoby.

THE INFLUENCE OF THE PHOSPHATE-CALCIUM RATIO AND OF HUMATES OF IRON ON CHLOROSIS IN LEMNA[1]

DALE HAROLD SIELING

From the Department of Chemistry, Iowa State College

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The iron humate contained in the alkaline extract of soils was studied in order to find its influence upon the growth of *Lemna major* at different reactions, and to determine the effect of the humus substances upon the availability of iron for plant growth at these reactions. The optimum phosphate-calcium ratio in nutrient solutions was investigated for the growth of *Lemna major* in a wide range of hydrogen ion concentration, and Olsen's (6) correlation of this ratio with chlorosis was examined for plants grown under sterile conditions.

INFLUENCE OF IRON HUMATES ON GROWTH AND CHLOROSIS OF LEMNA

Olsen (6) reported that the addition of humus extracts of soil to nutrient solutions with neutral or alkaline reactions showed the same influence in the prevention of chlorosis and stimulation of growth of *Lemna major* that was observed when ferric citrate was used as the source of iron; this stimulation was attributed to the property of the organic matter to make iron available for assimilation by the plant in neutral or alkaline reactions. Similarly, Burk et al (1) reported that the stimulation of growth of azotobacter by humic acids was due to the iron content of the humic acids employed. In order to examine these influences on the green plant under sterile conditions, the reproduction rate of Lemna was determined for varying quantities of humus and iron.

Alkaline humus extract—the iron humate—was prepared by extracting a peat soil with a potassium hydroxide solution. The "iron humate" was purified by precipitating with hydrochloric acid and redissolving the resulting precipitate in potassium hydroxide. The iron content of this solution was determined colorimetrically (5) and the quantity of humus in each cc. of the solution was found by drying a measured quantity at 110°C.

Lemna, free from micro-organisms, were grown at different reactions in Clark's (4) sterile solution to which had been added various quantities of iron humate and ferric chloride to give different total iron: iron-humate ratios. The plants were grown for five weeks in these solutions under controlled environmental conditions—25°C. and 14½ hours exposure daily to mazda illumination of 200 foot-candles intensity—and were transferred to freshly prepared solutions twice weekly (3). The growth rate of each culture was determined graphically by the method reported by Clark (2).

In all cases the rates of growth of Lemna at pH 4.5-5.0 were depressed by adding iron humate to the nutrient solution. In nutrient solutions con-

[1] Original thesis submitted December. 1936. Doctoral thesis number 413.

taining 0.01 millimols of iron per liter and having iron-iron humate ratios of 0.001, 0.002, and 0.005, the rate of growth increased progressively as the pH increased from 4.5 to 7.0, and then decreased as the reaction of the solution became more alkaline; at pH 7.0 the growth rate in every case was much greater than for Lemna in inorganic media with the optimum pH of 4.5-5.0. All plants were normally green with these ratios at acid and neutral reactions, but showed slight chlorosis in the alkaline solutions; chlorosis became more pronounced in the alkaline reactions as the iron-iron humate ratio was increased. In cultures containing one fifth the quantity of iron (0.002 millimols per liter) with an iron-iron humate ratio of 0.001, optimum growth was obtained at pH 6.0; at the same concentration growth decreased and chlorosis increased as the reaction became more alkaline. When the iron was increased to 0.02 millimols per liter and the organic matter was added to give an iron-iron humate ratio of 0.001 the growth rates at pH 6.0, 7.0, and 8.0 were practically identical, although slight chlorosis developed at pH 8.0. Nutrient solutions which contained ferric chloride or ferric citrate in concentrations to give 0.01 millimols of iron per liter produced optimum growth response at pH 4.5-5.0; growth decreased and chlorosis increased as the reaction became more alkaline than pH 5.0.

It was shown in these experiments that under sterile conditions organic matter—the iron humate—was effective in preventing chlorosis and stimulating growth of Lemna in cultures of Clark's solution at pH 7.0, and that chlorosis soon developed in cultures containing no organic matter when the reaction was above pH 5.0. The same observation was made by Olsen (6) with Lemna in modified Knop's solution, but he also reported that, with a reaction of pH 8.0, the plants were again normally green and gave good growth in solutions containing no organic matter; maximum chlorosis and minimum growth appeared at pH 6.0-7.0. By reducing the phosphate-calcium ratio of the Knop's solution to one fifth the usual figure, Olsen was able to grow maize plants for 18 days without chlorosis developing at pH 6.0-7.0; maximum growth was obtained at pH 7.0. An attempt was therefore made to establish the phosphate-calcium ratio most suitable for the growth of Lemna at pH 6.0-7.0 in a number of sterile solutions.

INFLUENCE OF PHOSPHATE-CALCIUM RATIO ON THE GROWTH AND CHLOROSIS OF LEMNA

Lemna, free from micro-organisms, were grown at pH 4.0, 5.0, 6.0, and 7.0 in Knop's solution (identical with the solutions used by Olsen but sterilized) containing 2.54 millimols of calcium and quantities of phosphate varying from 1.10 to 0.055 millimols; the phosphate-calcium ratios varied from 0.432 (Olsen''s high ratio) to 0.022 (one quarter of Olsen''s low ratio). These cultures were carried on for a period of five weeks under controlled environmental conditions and the plants were transferred to freshly prepared solutions twice weekly. In all cases the plants developed normally at pH 4.0 and 5.0, but became cholorotic and gave poor growth at pH 6.0 and 7.0, regardless of the phosphate-calcium ratio: chlorosis developed more slowly in the cultures of low phosphate-calcium ratios.

Sterile Lemna were then grown in a series of Clark's solutions which had been modified to give a calcium concentration of 0.40 millimols per liter, and phosphate-calcium ratios varying from 0.40 to 0.0008. The initial reactions of the solutions were adjusted to pH 7.0 and the plants were transferred twice weekly to freshly prepared solutions for a period of five weeks. At the time of transfer the pH of each exhausted solution was determined by the glass electrode. All the plants in this series developed chlorosis when the pH of the exhausted solutions remained above 5.4, but were normally green when the pH fell below 5.4 (phosphate-calcium ratios of 0.064 to 0.0008). By renewing the solution daily the pH's of the solution were retained at values above 6.0 and chlorosis developed in all cultures regardless of the phosphate-calcium ratio of the solution.

In these sterile cultures with strictly inorganic media and under electric light, the pH controlled the chlorosis, and no phosphate-calcium ratio variations succeeded in producing normal plants in alkaline solutions.

Similar results were obtained with Lemna placed in non-sterile solutions. Plants were grown for 17 days in Clark's and Knop's solutions with the phosphate-calcium ratios from 0.40 to 0.08 at pH 4.8, 6.0, 7.0, and 8.0, transferred daily to fresh solutions. In all cases the plants became chlorotic at pH 7.4 and 8.0, and also at pH 6.0 unless the pH fell below 5.5 before the end of the day. No solution was found, either in Knop's or Clark's media with any variation of the phosphate-calcium ratio, under sterile or non-sterile conditions, which would grow Lemna free from chlorosis at neutral or alkaline reactions, without the addition of organic matter.

CONCLUSIONS

1. Iron humate was effective in the prevention of chlorisis of Lemna in neutral reactions, but not completely in alkaline reactions.

2. Iron humate depressed the growth of Lemna at pH values of 4.5 to 5.0.

3. The effectiveness of iron humate in promoting growth and preventing chlorosis of Lemna at neutral reactions was attributed to its power to make iron available for assimilation by the plant.

4. Maximum stimulaion of growth was obtained when the iron-iron humate ratios were within the limits of 0.001 and 0.005 and the concentration of iron was 0.01 millimols per liter.

5. Nutrient solutions, containing ferric chloride but no organic matter, caused chlorosis of Lemna at reactions more alkaline than pH 6.0, irrespective of the phosphate-calcium ratio and the presence or absence of micro-organisms.

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SOME PHYSIOLOGICAL PROPERTIES OF BENZOFURAN AND DIBENZOFURAN DERIVATIVES[1]

E. Westley Smith

From the Department of Chemistry, Iowa State College

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I. BENZOFURAN

The presence of a partially reduced benzofuran nucleus in the accepted structure of morphine suggested a study of the physiological properties of benzofuran and benzofuran derivatives in a manner similar to that carried out, for the same reason, on phenanthrene and dibenzofuran. A survey of the literature of benzofuran revealed that very little is definitely known of the nuclear orientation of benzofuran, or of benzofuran derivatives, though a considerable number of reliable ring closure syntheses are available for the preparation of benzofuran and benzofuran derivatives. This situation made necessary an investigation of the positions assumed by nuclear substituents of benzofuran and of its derivatives.

Bromine and chlorine add readily to the 2,3-linkage of benzofuran[2,3]. The treatment of the dibromide with alcoholic potassium hydroxide yields 3-bromobenzofuran and distillation of the dibromide at atmospheric pressure yields 2-bromobenzofuran[2]. Benzofuran nitrates in the 2- position[2].

EXPERIMENTAL

Benzofuran-2-carboxylic acid is best prepared by the addition of bromine to coumarin in carbon disulfide and treatment of the resulting 3,4-coumarin dibromide with aqueous sodium hydroxide solution. An intimate mixture of benzofuran-2-carboxylic acid, calcium oxide, and copper-bronze readily decarboxylates upon heating to give good yields of benzofuran.

The reduction of benzofuran with hydrogen and noble metal catalysts at moderate temperatures and low pressures gave 2,3-dihydrobenzofuran. It was shown to be identical with authentic 2,3-dihydrobenzofuran[4] by comparison of the picrates, m.p. 76°-77°. The high temperature-high pressure reduction of benzofuran with hydrogen and Raney nickel catalyst yielded perhydrobenzofuran, b.p. 171°-172°, n_{25D} 1.4635.

Attempts to prepare 2-amino- and 3-aminobenzofuran, and benzofuran-2- and -3-carboxylic acid from 2- and 3-bromobenzofuran failed. The iodination of benzofuran was also not successful.

The metalation of benzofuran with *n*-butyl-lithium oriented the entering group to the 2- position. This was shown by carbonating the benzofuryl-2-lithium to the known benzofuran-2-carboxylic acid. Acetylation

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[1] Original thesis submitted August, 1936. Doctoral thesis number 407.

[2] Stoermer and Kahlert, *Ber.*, 35, 1633 (1902).

[3] Stoermer, *Ann.*, 312, 237 (1900).

[4] Alexander, *Ber.*, 25, 2409 (1892).

of benzofuran with acetic anhydride and stannic chloride yields 2-acetylbenzofuran. The same compound was prepared by the action of chloroacetone upon sodium salicylaldehyde[5], and additional proof of structure obtained by oxidation to the known benzofuran-2-carboxylic acid.

Attempts to sulfonate benzofuran were not successful, but benzofuran-2-sulfonic acid was prepared by treating benzofuryl-2-lithium with sulfur dioxide and oxidizing the resulting benzofuran-2-sulfinic acid with hydrogen peroxide to the sulfonic acid. The *p*-toluidide was prepared as a derivative, m.p. 188°-189°.

Attempted mercuration of benzofuran with mercuric acetate resulted in an oxidation of the nucleus. Picric acid also appeared to oxidize benzofuran under certain conditions.

The bromination of benzofuran-2-carboxylic acid failed, but ethyl benzofuran-2-carboxylate readily brominated in the 5- position. The 5-bromobenzofuran-2-carboxylic acid prepared by the hydrolysis of the bromination product was compared with authentic 5-bromobenzofuran-2-carboxylic acid prepared by the action of aqueous potassium hydroxide upon 3,6-dibromocoumarin[6], and by the reaction between sodium 5-bromosalicylaldehyde and ethyl bromoacetate. Nitration of ethyl benzofuran-2-carboxylate also oriented the entering group in the 5- position. This was shown by the oxidation of the product to 5-nitrosalicylic acid, and also by comparison with the 5-nitrobenzofuran-2-carboxylic acid of Dey and Row[7]. Acetylation with acetyl chloride and aluminum chloride also yielded a 5- substituted derivative of ethyl benzofuran-2-carboxylate. The identiy of the ethyl 5-acetylbenzofuran-2-carboxylate, m.p. 112.5°-114.5°, was established by oxidation to 4-hydroxyisophthalic acid. Mercuration of ethyl benzofuran-2-carboxylate failed.

2-Acetaminophenylallyl ether, m.p. 51°-52°, and 2-acetamino-6-allylphenol, m.p. 115°, were prepared as intermediates for the synthesis of 2-methyl-7-acetamino-2,3-dihydrobenzofuran.

II. DIBENZOFURAN

EXPERIMENTAL

The sulfonation of *o*-biphenol with fuming sulphuric acid by Zehenter[8] yielded a dibenzofuransulfonic acid. A comparison of the *p*-toluidide of this product and the *p*-toluidide of authentic dibenzofuran-2-sulfonic acid[9], m.p. 232°-233°, showed them to be the same.

A number of failures to replace the sulfonic group of dibenzofuran-2-sulfonic acid by nitro, carboxylic, bromo or chloro groups are reported, as well as attempts to replace the sulfinic group by a nitro group in dibenzofuran-2-sulfinic acid. The failure to prepare dibenzofuran-2-sulfonic acid by ring closure synthesis is probably due to the ready hydrolysis of the sulfonic group under the experimental conditions used.

Dibenzofuran-2,8-disulfonic acid was sulfonated to yield a dibenzofurantetrasulfonic acid; and dibenzofuran-2-sulfonic acid, dibenzofuran-

[5] Stoermer, *Ber.*, **30**, 1711 (1897).

[6] Perkin, *J. Chem. Soc.*, **24**, 37 (1871).

[7] Dey and Row, *J. Chem. Soc.*, **123**, 3375 (1923).

[8] Zehenter, *J. prakt. Chem.*, **131**, 331 (1931).

[9] Gilman, Smith and Oatfield, *J. Am. Chem. Soc.*, **56**, 1412 (1934).

2,8-disulfonic acid, and dibenzofurantetrasulfonic acid were hydrolyzed with sulfuric or hydrochloric acid under varying conditions to yield dibenzofuran.

Alkali fusions of dibenzofuransulfonic acids yielded no hydroxydibenzofuran compounds. Chlorination and bromination of dibenzofuran-2,8-disulfonic acid gave products suspected of having a powerful vesicant action. Nitration of dibenzofuran2,8-disulfonic acid gave a nitrodibenzofuran-2-sulfonic acid. The sulfonic group of this compound could not be removed by hydrolysis to yield a nitrodibenzofuran. Sodium dibenzofuran-2-sulfonate was readily mercurated with mercuric acetate and treated with sodium chloride to yield sodium chloromercuridibenzofuran-2-sulfonate. The chloromercuri group was replaced with bromine.

The Gattermann-Koch reaction for the introduction of an aldehyde group did not proceed with dibenzofuran, but dibenzofuryl-2-aldehyde, m.p. 71°-72°, was prepared from dibenzofuran-2-nitrile. An excellent method for the preparation of dibenzofuran-2,8-dicarboxylic acid from 2,8-dibromobenzofuran, *via* the dinitrile, was developed.

Lead tetra-acetate had no action on dibenzofuran, and oxidized tetrahydrodibenzofuran in part to dibenzofuran. Mercuric acetate also acted as an oxidizing agent with tetrahydrodibenzofuran though some unidentified mercuration products were also produced.

A number of reagents were used in an endeavor to oxidize 7-nitro- and 7-acetyl-1,2,3,4-tetrahydrodibenzofuran to products of known structure before the reaction was successfully carried out by means of bromine in glacial acetic acid[10].

[10] Gilman, Smith and Cheney, *J. Am. Chem. Soc.*, 57, 2095 (1935).

THE OCCURRENCE OF PHOSPHOGLYCERIC ACID IN THE BACTERIAL DISSIMILATION OF GLUCOSE[1]

ROBERT W. STONE

From the Department of Bacteriology, Iowa State College

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Recent investigations on the biological breakdown of glucose have placed in question the intermediary role of methylglyoxal. The work of Embden, Meyerhof and others has produced convincing evidence that glucolysis in yeast and muscle involves phosphorylated intermediaries, such as phosphoglyceric acid, in place of methylglyoxal. The work reported here was undertaken to determine whether phosphoglyceric acid occurs in bacterial glycolysis.

In order to determine some of the variables affecting the formation of phosphoglyceric acid, a number of experiments were carried out with yeast. Fresh yeast was incubated at 37°C. in mixtures of phosphate buffer (pH 6.8), glucose, hexosediphosphate, acetaldehyde, sodium fluoride, magnesium chloride and toluene. The phosphoglyceric acid was isolated by precipitation as the barium salt. The acid probably arises by the following reaction, for which hexosediphosphate acts as a catalyst:

$$\text{(1)} \quad \text{glucose} + 2\,H_3PO_4 + 2\,CH_3CHO \rightarrow 2 \text{ triosephosphate} + 2\,CH_3CHO \rightarrow 2 \text{ phosphoglyceric acid} + 2\,CH_3CH_2OH.$$

It was found that variations in time and the concentrations of acetaldehyde and fluoride markedly influence the formation of the phosphate ester, and that there exist complex interactions among these variables. There was a significant correlation between uptake of inorganic phosphate and yield of phosphoglyceric acid salt. In working up the reaction mixtures and unknown yellow amorphous precipitate was found. The quantity of this precipitate correlated inversely with the yield of phosphoglyceric acid and phosphate uptake.

For the experiments with bacterial glycolysis, the organisms were cultured in appropriate liquid media and centrifuged out, and the result ing moist cell paste was used as in the above method for yeast. Phosphoglyceric acid was isolated from all the bacteria studied except *Clostridium butylicum*. The species include *Propionibacterium pentosaceum*, *P. arabinosum*, *Escherichia coli*, *Aerobacter indologenes*, *Bacillus subtilis*, *Serratia marcescens*, *Lactobacillus plantarum*, *L. pentoaceticus*, *Streptococcus paracitrovorus* and *Azotobacter vinelandii*. Ease of formation of the acid was roughly in the order named, although the optimal conditions for *B. subtilis*, *S. marcescens* and *A. vinelandii* were not determined. The uptake of phosphate and quantity of phosphoglyceric acid formed showed a significant correlation. It is noteworthy that a great deal more phosphate was esterified than could be accounted for in the phosphoglyceric acid

[1] Original thesis submitted December, 1936. Doctoral thesis number 415.

obtained. The excess phosphate may have been held as triose or hexose esters, probably precursors of phosphoglyceric acid. This supposition is supported by the fact that if the mixtures were allowed to stand for several days at 5°C. after the regular incubation time, there was a substantial increase in the amount of acid that was isolated, with no corresponding increase in phosphate uptake.

Although no phosphoglyceric acid was obtained with *Cl. butylicum*, large amounts of a yellow precipitate occurred. This unknown substance appeared to be similar if not identical to the precipitate formed by yeast. However, the quantities of the precipitate formed by *Cl. butylicum* correlated directly with the phosphate uptake, while yeast with the correlation was an inverse one. The unknown compound dried to a glue-like mass, and was very resistant to hydrolysis.

Several of the organisms which form phosphoglyceric acid readily were chosen for study in relation to the effect of aging, aeration, etc. It was found that if the organisms were centrifuged from the culture medium, kept at 5°C. in a covered container and used subsequently to produce phosphoglyceric acid, there was a noticeable decrease in yield after one day and a corresponding decrease thereafter. Cells dried in air formed some ester under favorable conditions, but did not compare with fresh cells in this regard. If the reaction mixtures were aerated during the incubation period, the yield of acid was measurably increased, while if the experiment was carried out in the presence of nitrogen a decrease in yield usually resulted. The effect of aeration may have been due to the action of oxygen in supplementing acetaldehyde as a hydrogen acceptor.

The use of such compounds as hexosediphosphate, acetaldehyde, sodium fluoride and toluene aided greatly in obtaining phosphoglyceric acid. However, it was shown that no one of these is indispensable. In the case of hexosediphosphate it was found that if phosphoglyceric acid can be isolated with the hexose ester present, it can be isolated from glucose alone. It was necessary to precede the regular incubation time by a phosphorylation period in which the organisms, glucose, phosphate buffer and toluene were present. After a few hours there was sufficient hexosediphosphate formed from the glucose to catalyze reaction 1; and at this point sodium fluoride and acetaldehyde were added to cause the accumulation of phosphoglyceric acid.

Acetaldehyde is not necessary for isolation of phosphoglyceric acid, and may be replaced by other hydrogen acceptors. In fact, the ester was obtained in small quantities with no added hydrogen acceptor present, as in equation 2.

(2) glucose + 2 $H_3PO_4 \rightarrow$ 2 triosephosphate $\rightarrow$ α-glycerophosphoric acid + phosphoglyceric acid.

With the propionic acid bacteria it was found that pyruvic acid could replace acetaldehyde and give increased yields of phosphoglyceric acid. Propaldehyde also acted as an acceptor, but was not so effective as acetaldehyde. Acetylmethylcarbinol and lactic acid had no effect. However, with *E. coli* and *A. indologenes* acetylmethylcarbinol gave much larger yields than acetaldehyde, while pyruvic acid was slightly inferior to the latter.

Toluene was replaced by xylene or chloroform with no decrease in formation of phosphoglyceric acid. If toluene or similar reagents were omitted it was still possible to obtain some ester under favorable conditions, accompanied by phosphate uptake. This fact is evidence that phosphorylation is concerned with glucolysis in the living cell and not limited to toluene-treated or dried organisms as some workers have claimed. In the presence of toluene and the absence of sodium fluoride it was possible to obtain a very small amount of phosphoglyceric acid. In the absence of both toluene and fluoride or like reagents no ester was isolated. Apparently some abnormal treatment is necessary in order to break into the reactions of glycolysis and cause sufficient accumulation of a phosphorylated intermediary for its isolation.

This invetigation has shown that phosphoglyceric acid occurs under a variety of conditions in the glycolysis of all the bacteria studied, with the exception of *Cl. butylicum.* Therefore, many bacteria must possess the necessary enzyme equipment to produce phosphoglyceric acid, and this compound may well be an intermediary in their glycolytic processes. The present bacterial fermentation schemes should be reinvestigated with a view to including phosphoglyceric acid.

CERTAIN BIOLOGICAL STUDIES ON PHYLLOPHAGA (COLEOPTERA: SCABABAEIDAE) IN IOWA[1]

BERNARD V. TRAVIS

From the Department of Zoology and Entomology, Iowa State College

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White grubs belonging to the genus Phyllophaga Harris include a large number of beetles popularly known as "June-bugs" or "May-beetles" whose larvae are the common white grubs of the fields. From an economic point of view, the depredations of both the larvae and adults are well known.

The genus is indigenous to the Western Hemisphere and ranges from the Hudson Bay south to Argentine, including the West Indian Islands. Thirty-four species have been recorded from Iowa. These are listed in the order of numerical numbers as determined by records of specimens collected at random: *Phyllophaga hirticula* (Knoch), *P. implicita* (Horn), *P. fusca* (Froel.), *P. tristis* (Fab.), *P. futilis* (Lec.), *P. rugosa* (Mels.), *P. inversa* (Horn), *P. crassissima* (Blanch.), *P. horni* (Smith), *P. anxia* (Lec.), *P. drakii* (Kirby), *P. fraterna* Harris, *P. ilicis* (Knoch), *P. micans* (Knoch), *P. crenulata* (Froel.), *P. marginalis* (Lec.), *P. prunina* (Lec.), *P. congrua* (Lec.), *P. fervida* (Fab.), *P. nitida* (Lec.), *P. balia* (Say), *P. bipartita* (Horn), *P. fosteri* (Burm.), *P. corrosa* (Lec.), *P. vilifrons* (Lec.), *P. barda* (Horn), *P. lanceolata* (Say), *P. longitarsa* (Say), *P. gracilis* (Burm.), *P. quercus* (Knoch), *P. spreta* (Horn), *P. ephilida* (Say), and *P. hirtiventris* (Horn). It is worthy of special note that the first six species of the foregoing list are the dominate forms in all three broods.

In an attempt to determine the efficiency of insecticides as a practical measure of poisoning adults, several different kinds of stomach poisons were dusted or sprayed on foliage normally used by the beetles as food. The treated foliage was then offered the following species of beetles: *P. implicata, P. hirticula* and *P. rugosa*.

The materials used as dusts were applied in the following proportions: 1.5 pounds (light application), 3 pounds (moderate application), 4.5 pounds (heavy application). All poisons except sodium fluosilicate contained two parts by weight of the commercial insecticide to three parts of finely powdered bentonite. The sodium fluosilicate was a proprietary product. The lead and calcium arsenate sprays were used at the rate of one or two pounds of the poison to 50 gallons of water . The latter compounds were also tested as dusts on trees in the field.

Sulphur, whiting substance, hydrated lime, diatomaceous earth, kaolin, and lead arsenate which have little or no toxic effect on adults were used in parallel experiments without poisons.

One-half of the beetles were offered poisoned foliage for one night only, whereas the remaining half received the treated leaves for two nights in succession.

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The percentages of the beetles dead after feeding one or two nights upon leaves dusted with applications of the poison were as follows:

Lead arsenate—light, 74.90; moderate, 81.95; heavy, 88-98.

Calcium arsenate—light, 15-52; moderate, 36-70; heavy, 51-84.

For leaves sprayed with the poison, the percentages were as follows:

Lead arsenate—one pound, 12-40; 2 pounds, 62-70.

Calcium arsenate—one pound, 34-48; 2 pounds, 28-38.

Magnesium arsenate and sodium fluosilicate were not highly toxic to the beetles.

When used alone the diluent dust compounds caused no significant mortalities. Highest mortalities were obtained when poisoned food was offered for two nights in succession. Experimental evidence seemed to indicate that not all the beetles fed in the cages when the poisoned foliage was offered for only one night.

A median lethal dose for paris green, cuprous cyanide, arsenious oxide, and acid lead arsenate were, respectively, 0.03, 0.04, 0.06, and 0.12 milligrams per gram body weight. Many individuals in these tests regurgitated copiously after feeding upon the poisoned folaige. Only one of the beetles, and this one fed on leaves treated with paris green, survived after regurgitation. Nearly half the females laid fertile eggs after having ingested foliage with lead arsenate. The 50 per cent survival point was reached in about 8 hours for paris green and cuprous cyanide; in about 13 hours for arsenious oxide; and in about 20 hours for lead arsenate.

White grub migration and hibernation were studied in Iowa during the years 1930-1934. The records for 1930-1932 were from Decatur County, and for 1933-1934 from Floyd County. The dates for the initial start and completed downward migration were as follows: 1930, Oct. 15-17 to Nov. 14; 1932, Oct. 23 to Nov. 17; 1933, Oct. 30 to Nov. 16. The dates for the upward migration were: 1931, April 7-16 to May 19; 1933, April 1-9 to May 10; 1934, April 27 to May 5, incomplete. During the latter year many larvae never migrated upward in the spring to feed before pupating in their hibernation cells.

Larvae collected at fall migration time were liberated in an unplowed field to determine the rate of downward migration. These individuals were then dug up at regular intervals to determine the rate and distance traveled. The migration was almost vertically downward. The rates of the downward movement were as follows: 24 hours, 1-10 inches, mean 5.3 inches; 48 hours, 4-15 inches, mean 9.4 inches; 72 hours, 2-20 inches, mean 11.9 inches; 96 hours, 5-18 inches, mean 11.1 inches.

The hibernation depths of the larvae during 1930 were: bluegrass, 7-22 inches, mean 14.4; timothy, 13-24 inches, mean 15.5; corn, 10-22 inches, mean 19.1. In 1932 the depths were: : bluegrass, 9-24 inches, mean 14.5. In 1933 the depths were: bluegrass, 9-30, mean 17.9; corn, 1-30 inches, mean 17.5 inches.

The pupation depths in 1930 were: 5-15 inches, mean 10.9 inches; in 1931, 3-17 inches, mean 11.5 inches; in 1934, 2-31 inches, mean 19.1 inches.

In 1931 the first prepupa was found on July 2, the first pupa July 24, and the first adult August 20. In 1934 the first prepupa was found June 28, the first pupa July 6, and the first adult August 4.

The parasites, Tiphia sp. and Elis sp., were encountered many times during these studies. The pupation depths for these insects were: 1930,

1-16 inches, with a mean of 7.3 inches; 1931, 3-17 inches, with a mean of 12.3 inches.

Observations on adult beetles indicates that the starting time of the evening flight varies from 7:55-8.21 p. m., the morning flight from 3:25-4:15 a. m. The evening flight extends over a period of about 50 minutes, the peak being 15-20 minutes after the first beetle issued from its hiding place.

The habits of the diurnal beetle, *P. lanceolata,* was studied in some detail. This species appears to feed on a larger number of plants than other species studied. The males may be attracted by crushing a female, or by the vapor of the aromatic compound isoamylamine.

The females of *P. lanceolata* contained an average of 27 eggs when dissected on June 26, 1934. Laboratory tests showed that this species deposited eggs most freely in jars containing air-dried soil to which 25 per cent by weight of water had been added.

DIBENZOFURAN AND PHENOTHIAZINE DERIVATIVES[1]

PAUL RAYMOND VAN ESS

From the Department of Chemistry, Iowa State College

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I. DIBENZOFURAN DERIVATIVES

In connection with a series of studies on the orientation of dibenzofuran derivatives[2], the writer investigated the bromination reaction of 2- and 4-hydroxy- and 4-acetaminodibenzofurans.

I Bromination of 2-hydroxydibenzofuran yielded principally 1-bromo-2-hydroxydibenzofuran (m.p. 123-123.5°), and some of the isomeric 2-hydroxy-3-bromodibenzofuran isolated as 2-methoxy-3-bromodibenzofuran (m.p. 172°). Methylation of 1-bromo-2-hydroxydibenzofuran yielded 1-bromo-2-methoxydibenzofuran (m.p. 117-118°).

Bromination of 2-methoxydibenzofuran yielded principally 2-methoxy-3-bromodibenzofuran and some of the isomeric 1-bromo-2-methoxydibenzofuran. The structure of 2-methoxy-3-bromodibenzofuran was proved by its synthesis from the previously established 2-amino-3-bromodibenzofuran[2c] through diazotization, replacement of the diazonium group by hydroxyl to yield 143-144° melting 2-hydroxy-3-bromodibenzofuran, and methylation.

The reaction of allyl bromide with the Grignard reaction of 1-bromo-2-methoxyldibenzofuran yielded 1-allyl-2-methoxydibenzofuran (m.p. 67-68°). The Claisen allyl ether arrangement of 2-allyloxydibenzofuran yielded 83° melting 1-allyl-2-hydroxybenzofuran, whose methylated derivative (m.p. 67-68°) was found identical with the reaction product of allyl bromide with the Grignard reagent of the 117-118° melting 1-bromo-2-methoxydibenzofuran.

Evidence for the structure of 1-allyl-2-methoxydibenzofuran, and hence of 1-bromo-2-hydroxydibenzofuran, rests upon the assumption that the allyl ether rearrangement, as shown by Claisen[3], is a reliable *ortho* rearrangement. Thus, the phenol obtained by pyrolysis of 2-allyl-oxydibenzofuran can be only 1- or 3-allyl-2-hydroxydibenzofuran; but its 67-80° melting methylated derivative was also obtained by conversion, not from the established 172° melting 2-bromo-3-methoxydibenzofuran, but from the methyl ether of the 123-123.5° melting monobromination product of 2-hydroxybenzofuran. Hence, in the allyl ether rearrangement of 2-allyloxydibenzofuran, the possibility of migration of the allyl group to the 3-position is excluded and only the 1-position can be involved.

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[1] Original thesis submitted August, 1936. Doctoral thesis number 406.

[2] a Gilman, Smith and Oatfield, *J. Am. Chem. Soc.*, **56**, 1412 (1934).
b Gilman and Young, *ibid.*, **56**, 1415 (1934).
c Gilman, Brown, Bywater and Kirkpatrick, *ibid.*, **56**, 2473 (1934).
d Gilman, Bywater and Parker, *ibid.*, **57**, 885 (1935).
e Gilman and Young, *ibid.*, **57**, 1121 (1935).
f Gilman, Smith and Cheney, *ibid.*, **57**, 2095 (1935).

[3] Claisen, *Ber.*, **45**, 3159 (1912); Claisen and Eisleb, *Ann.*, **401, 21** (1913); Claisen, Eisleb and Kramers, *Ann.*, **418**, 69 (1919).

2-Methoxy-8-bromodibenzofuran (m.p. 92.5°) was prepared by ring closure of 2-amino-4-bromo-4′-methoxydiphenyl ether.

Bromination of 4-hydroxydibenzofuran yielded a monobrominated derivative (m.p. 151.5-152°), whose structure is certainly 1- or 9-bromo-4-hydroxydibenzofuran, and most probably 1-bromo-4-hydroxydibenzofuran. Methylation of 151.5-152° melting 1-bromo-4-hydroxydibenzofuran yielded 1-bromo-4-methoxydibenzofuran (m.p. 97-98°), identical with the bromination product of 4-methoxydibenzofuran.

Bromination of 4-acetaminodibenzofuran occurred smoothly, giving an excellent yield of 1-bromo-4-acetaminodibenzofuran (m.p. 228°), very little if any isomer formation being involved. 1-Bromo-4-aminodibenzofuran (m.p. 119-120°) was obtained by alkaline hydrolysis of 1-bromo-4-acetaminodibenzofuran. Conversion of the 1-bromo-4-amine to 1-bromo-4-hydroxydibenzofuran proves that bromination of 4-acetamino-, 4-hydroxy-, and 4-methoxydibenzofuran takes place in the same position.

Deamination of 1-bromo-4-aminodibenzofuran yielded 1-bromodibenzofuran (m. p. 67°), the first reported 1-monosubstituted dibenzofuran. The structure of 1-bromodibenzofuran rests upon its dissimilarity from the previously established 2-, 3-, and 4-bromodibenzofurans. 1-Carboxy-, 1-carbomethoxy-, 1-hydroxy-, 1-amino-, and 1-acetaminodibenzofurans were prepared from 1-bromodibenzofuran. In each instance, the 1-substituted derivative differed from the corresponding 2-, 3-, and 4-substituted derivatives.

The synthesis of 1-bromodibenzofuran from the 119-120° melting 1-bromo-4-aminodibenzofuran proves that the bromination of 4-acetaminodibenzofuran involves the 1- or 9-positions; 1- or homosubstitution is strongly indicated by consideration of the pronounced *ortho* and *para* directing influence of the acetamino group.

Nitration of 1-carbomethoxydibenzofuran (m.p. 63°) yielded 3- or 7-nitro-1-carbomethoxydibenzofuran (m.p. 216°). Nitration in the 3- or 7-position was proved by isolation of 3-nitrodibenzofuran upon decarboxylation of the nitro-acid.

II. PHENOTHIAZINE DERIVATIVES

Continuing a series of studies on the metalation of unsaturated heterocycles [2b,1e,4], *n*-butyl-lithium metalation of 10-ethylphenothiazine[5] and subsequent carbonation gave a small purified yield of monocarboxy-10-ethylphenothiazine (m.p. 178-179°). The structure of this monocarboxy-acid was not proved, but the possibility of 3-metalation was excluded by the preparation of 3-carboxy-10-ethylphenothiazine which melted at 197.5-198.5° and was dissimilar from the 178-179° melting isomer.

3-Carboxy-10-ethylphenothiazine was prepared by carbonation of the Grignard reagent of 3-iodo-10-ethylphenothiazine (m.p. 126-127°), which, in turn, was synthesized from Finzi's 3-acetoxymercuri-10-ethylphenothiazine[6].

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[4] Gilman and Kirby, *J. Org. Chem.*, **1**, 146 (1936).

[5] New international rules for numbering ring systems used. Patterson, *J. Am. Chem. Soc.*, **47** 540 (1925); *ibid.*, **50**, 3074 (1928).

[6] Finzi, *Gazz. chim. tal.*, **62**, 175 (1932) (*C. A.* **26**, 4338, (1932).

By analogy with the metalation of 5-ethylcarbazole *ortho* to the tertiary nitrogen atom[4], metalation of 10-ethylphenothiazine *ortho* to the nitrogen atom, or in the 1-position, is indicated.

In comparison with dibenzofuran [2b,2e], 10-ethylpenothiazine undergoes *n*-butyl-lithium metalation with difficulty, but mercurates with ease.

FURANIC AND RELATED HETEROCYCLIC DYES AND COLORED MOLECULES[1]

MARIAN WESTON VAN ESS

From the Department of Chemistry, Iowa State College

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I. FURAN DERIVATIVES

In a study of 5-nitro-2-furoic acid, Hill and White[2] found that this acid reacted with two equivalents of aniline hydrochloride in aqueous sodium acetate solution to give a red product, which was resolved into two constituents. The main fraction, a yellow compound, $C_{16}H_{13}ON_3$, melted at 232° with decomposition when rapidly heated. The red minor product, $C_{17}H_{13}O_3N_3$, melted at 216° with decomposition. The yellow body on reduction gave ammonia, aniline, and succinanil. The findings of these investigators were checked in this laboratory by R. J. Vander Wal, who suggested that the yellow dye might be 1-phenyl-5-benzeneazo-2-pyrrolol. He also prepared the yellow compound from 2-nitrofuran. The writer continued the investigation from this point.

Vander Wal's proposed structure for the yellow compound was confirmed by synthesizing the compound by the oxidation of 1-phenyl-2-benzeneazopyrrole. A monoacetyl derivative was prepared; it melted at 197°. The yellow compound was hydrolyzed by prolonged refluxing with hydrochloric acid, but only one product, aniline, was identified.

The red compound was shown to be non-acidic. It did not form a quinoxaline derivative with o-phenylenediamine. The orange monoacetyl derivative melted at 195°. Aniline was identified among the reduction products of the red compound, but more significant was the hydrolysis with aqueous alkali to give aniline and 1-phenyl-3, 5-dicarboxypyrazole in good yields. The 1-phenyl-3,5-carboxypyrazole was thought to be produced by a secondary reaction from the monophenylhydrazone of α,α'-diketoglutaric acid, since the pyrazole nucleus could not be present in the non-acid original compound. The red compound was assigned the structure, 1-phenyl-2,3,5,6-piperidinetetrone 3-phenylhydrazone, since this formula represents the anil of α-phenylhydrazono-α'-ketoglutaric acid.

The mechanism of the reaction by which the red and yellow compounds were formed from 5-nitro-2-furoic acid was also studied. The first step is apparently the formation of the aniline salt of nitrofuroic acid, since this salt precipitated out if the reaction mixture was too concentrated. The salt also serves satisfactorily as starting material for the reaction. The second step, by analogy with the reaction of furfural and other furan derivatives with amines, is thought to be ring scission in which the oxygen bridge is broken by the addition of aniline, the anilino group going to the carbon holding the nitro group, and the hydrogen forming an hydroxyl group on the carbon holding the carboxyl group. The nitro group is then

[1] Original thesis submitted August, 1936. Doctoral thesis number 405.

[2] Hill and White, *Am. Chem. J.*, 27, 193 (1902).

presumably replaced by hydroxyl, possibly similar to the loss of halogen when 5-bromo- or 5-chlorofufural undergoes ring scission with aniline[3]. The aliphatic compound thus formed is then thought to undergo ring closure in two fashions to give 1-phenyl-2-hydroxy-5-carboxypyrrole and 1-phenyl-3,5-dihydroxy-2-pyridone (or tautomeric forms of these compounds). These two heterocyclic compounds may then couple with benzenediazonium hydroxide in a manner similar to the established behavior of like compounds to give the yellow and the red compounds, respectively. The presence of benzenediazonium chloride in the reaction mixture was demonstrated by carrying out the reaction in the presence of β-naphthol and isolating from the products the known 1-benzeneazo-2-hydroxynaphthalene.

II. DIBENZOFURAN DERIVATIVES

As a part of a general study of the orientation of substituents in the dibenzofuran nucleus, the writer undertook the investigation of the coupling reactions of the 2-, 3-, and 4-hydroxibenzofurans with benzenediazonium chloride. I-Benzeneazo-2-hydroxydibenzofuran crystallized in red needles, insoluble in alkali, melting at 165.5-166°. 2-Benzeneazo-3- hydroxydibenzofuran was also insoluble in alkali, but golden brown in color, and melted at 177-178°. The yellow-orange 1-benzeneazo-4-hydroxydibenzofuran was soluble in alkali and had a melting point of 174-175°. Th structures of these dyes were established by reduction to the corresponding amino derivatives, which were converted to the known hydroxy-bromo compounds by the Sandmeyer reaction.

As a continuation of the work of Hayes[4], the writer further examined the orientation of substituents upon nitration and bromination of 4-carbomethoxydibenzofuran. Hayes' 2-(or 8)-bromo-6-carbomethoxydibenzofuran was definitely established as 2-bromo-6-carbomethoxydibenzofuran by comparing it as the ester and as the acid with a sample prepared by ring closure of 2-amino-6-carboxy-4′-bromodiphenyl ether. 2-Bromo-4-methyldibenzofuran, m.p. 106-106.5°, was also prepared by a similar ring closure, but was not successfully oxidized to 2-bromo-4-carboxydibenzofuran.

The structure of Hays' 3-(or 7-)nitro-6-carbomethoxydibenzofuran was proved to be 3-nitro-6-carbomethoxydibenzofuran by establishing a structural relationship with the now known 2-bromo-6-carbomethoxydibenzofuran. The same compounds, later shown to be 2-bromo-3-acetamino-6-carbomethoxydibenzofuran, m.p. 247-247.5°, was prepared from both of these esters, thus proving the nitro-ester to be 3-nitro-6-carbomethoxydibenzofuran. 2-Bromo-6-carbomethoxydibenzofuran was nitrated to give nitro-2-bromo-6-carbomethoxydibenzofuran, m.p. 205-206°, which by reduction and acetylation gave the previously mentioned bromo-acetamino-ester. This same nitro-2-bromo-6-carbomethoxydibenzofuran was hydrolyzed to the corresponding acid, which when decarboxylated also lost its halogen atom to yield 3-nitrodibenzofuran. Since it was known by the relationship to Hayes' nitro-ester that the bromo-nitro-ester was either 2-bromo-3-nitro-6-carbomethoxydibenzofuran or 2-bromo-7-nitro-6-carbomethoxydibenzofuran, it was certain that decarboxylation involved tran-

[3] Hewlett, Doctoral Dissertation, Library, Iowa State College, 1930.

[4] Hayes, Thesis, Library, Iowa State College, 1934.

siently either 2-bromo-3-nitro- or 2-bromo-7-nitrodibenzofuran. It was then shown that under the conditions of decarboxylation, 2-bromo-7-nitrodibenzofuran was entirely stable, whereas 2-bromo-3-nitrodibenzofuran was converted to 3-nitrodibenzofuran. Hence it was concluded that the bromo-nitro-ester was 2-bromo-3-nitro-6-carbomethoxydibenzofuran, and the bromo-acetamino-ester prepared from it by reduction and acetylation was 2-bromo-3-acetamino-6-carbomethoxydibenzofuran. Hayes' 3- (or 7-) nitro-6-carbomethoxydibenzofuran was reduced to the amine and acetylated to give a product melting at 245-246°, which upon bromination gave 2-bromo-3-acetamino-6-carbomethoxydibenzofuran.

The findings of this study are in harmony with the general rules of orientation in the dibenzofuran series, that heterosubstitution will take place when one ring is previously occupied by a *meta*-director, or to a somewhat lesser extent when a halogen substituent is present; and that in heterosubstituted dibenzofurans, the second substituent, be it like or unlike the first, is directed to the positions involved in the monosubstitution of the nucleus itself.

THE BUTYL-ACETONIC FERMENTATION OF THE JERUSALEM ARTICHOKE[1]

Ray T. Wendland

From the Department of Chemistry, Iowa State College

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Levulose is readily fermentable by *Cl. acetobutylicum* producing the usual solvents, butanol, ethanol and acetone, and, since the acid hydrolyzed juices of the artichoke tuber contain significant amounts of levulose, attempts were made to produce successful butyl-acetonic fermentations of these artichoke hydrolyzates.

While the fresh tubers of the artichoke were not readily available, there was access to a large supply of dried artichoke chips which constituted a satisfactory source of the desired sugars. For the purpose at hand these chips were either extracted with hot water in a diffusion battery, or were ground and used directly in the fermentation media. To facilitate storage of the diffusion juice, large batches were concentrated by vacuum evaporation. Mold growth on the surface of the syrup could be prevented by the introduction of carbon dioxide into the storage vessel.

Since the butyl organisms hydrolyze starch so readily, it was thought that they might also hydrolyze the levulose polysaccharides of the artichoke juices. To test this possibility, experiments were arranged in which both the diffusion juices and the sludge were varied, and several cultures of butylacetone organisms producing satisfactory yields of solvents in corn mash were used for inoculation of the media. The fermentations resulting were not vigorous and the solvent yields were inappreciable. It was thus apparent from these experiments that the polysaccharides of the artichoke cannot be fermented directly by the butyl organisms.

Next in order of the investigation was the fermentation of the various hydrolyzed juices. Hydrolysis of the diffusion juice and the chip sludge was accomplished by the use of hydrochloric or sulfuric acid, according to Eichinger's method (1). These various hydrolyzates were diluted so that the reducing sugar concentration ranged from 2 to 10 per cent, the liquors were sterilized in Erlenmeyer flasks, and then inoculated with butyl cultures growing in 5 per cent corn mash. The fermentatitive activity was better than that observed in the unhydrolyzed juices, but the solvent yields, as determined by the specific gravity measurement of the distillate, and by the method of Christensen and Fulmer (2) were only 20 to 30 per cent of the sugar present.

These results led to the belief that the artichoke juices do not contain the nitrogenous materials which the butyl organisms require for normal solvent production. Accordingly, various protein supplements in the form of corn mash and soy bean meal were added to the hydrolyzates. The fermentations of these protein supplemented hydrolyzates were much more active than the others described above. Table 1 shows the composition of the media and the solvent yields obtained by replacement of corn mash

[1] Original thesis submitted June, 1937. Doctoral thesis number, 436.

by various amounts of artichoke hydrolyzate. The solvent yields ranged from 36.5 to 39.6 per cent and there is no significant decline in solvent yields up to the point where the levulose of the hydrolyzate constitutes 72.5 per cent of the total sugar.

TABLE 1. *Fermentation of hydrolyzates supplemented by corn mash*

	Flasks				
	1	2	3	4	5
Amount of corn meal, grams	220.0	180.0	140.0	100.0	60.0
Artichoke hydrolyzates, cc.	0	380.0	760.0	1140.0	1250.0
Final volumes, cc.	3325.0	3390.0	3400.0	3345.0	3400.0
Dextrose equivalent, g.	134.4	109.96	85.5	61.1	36.6
Levulose in the juice, g.	0.0	28.1	56.2	84.3	92.2
Total sugar, g.	134.4	138.06	141.7	145.4	128.8
Total solvents, g.	53.2	52.78	52.60	53.00	48.62
Yield of solvents from total sugar, per cent	39.6	38.2	37.2	36.5	37.8

In subsequent experiments the quantity of hydrolyzate was increased to the point of 85 to 90 per cent replacement of the corn mash before there was any appreciable decrease in the percentage conversion of total sugar to solvents.

The results obtained after the addition of soy bean meal to the hydrolyzates are given in table 2. The first 9 flasks received 650 cc. of hydrolyzate, equivalent to 22.75 g. of sugar, together with the amounts of soy bean meal indicated. Number 10 was the control and contained 45 g. of corn equivalent to 27.90 g. of dextrose. Solvent yields were determined by the specific gravity measurements of the distillates, and also by the standard acetone titration.

TABLE 2. *Influence of soy bean meal on the fermentation of artichoke hydrolyzate*

Flask No.	Soy bean content		Solvents g. per 100 cc. of distillate		Total solvents g. per flask	Percentage yield from the sugar
	g.	Pctg.	Acetone	Total		
1	0	0	0.51	1.61	4.83	21.2
2	3	0.37	0.72	1.79	5.69	25.1
3	5	0.62	0.95	2.41	7.36	32.4
4	8	0.95	1.09	2.84	8.65	38.0
5	12	1.50	1.11	2.80	8.59	37.3
6	15	1.88	1.09	2.87	8.69	38.2
7	20	2.50	1.14	2.86	8.64	38.0
8	25	3.10	1.01	2.76	8.40	36.9
9	30	3.70	0.97	2.68	8.15	35.7
10	Corn mash control		1.18	3.80	11.31	40.6

The solvent yields reached a maximum when the soy bean concentration was about 0.9 per cent. The yields were of the same order up to

about 2.5 per cent soy bean concentration, after which they diminished slightly.

In order ot check the routine analytical methods, and prove that butanol, ethanol and acetone are actually the products formed, large scale fermentations were carried out to provide sufficient liquor for the separation and identification of the volatile products. Round bottom flasks of 22-liter capacity were used, and the solvents from the fermentation of hydrolyzates with corn mash, and with soy bean meal were separated by distillation and fractionation, and then characterized by the preparation of derivatives. In each case the fraction boiling at 55° to 56° was proven to be acetone through its 2, 4, dinitro phenylhydrazone, and that boiling at 115° to 116° was shown to be butanol through its 3, 5 dinitro benzoate.

Subsequent work, using 15 to 20 liters of fermenting soy bean hydrolyzate in the large flasks, showed that the concentration of reducing sugar could be increased to about 5.5 per cent before there was any appreciable decline in solvent yields. In a particular case where the sugar concentration was 5.25 per cent and the soy bean content 0.85 per cent, the solvent yield from the sugar was 36.8 per cent.

These results show, (1) that the polysaccharides of the artichoke tuber cannot be fermented directly by the butyl organisms, (2) that after hydrolysis of the polysaccharides the yield of solvents from fermentation is 20 to 30 per cent of the sugar present, and (3) that the addition of corn mash or soy bean meal to the hydrolyzates increases the yields of solvents to 36 to 39 per cent. The latter figures are those commonly observed in the commercial butyl-acetonic fermentation of corn mash.

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A STUDY OF THE PIEZO-ELECTRIC OSCILLATIONS OF QUARTZ PLATES[1]

ROBERT G. WILSON

From the Department of Physics, Iowa State College

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Very little theoretical work has been done in the field of piezo-electric oscillations. The problem is so complicated by the fact that one is dealing with an anisotropic medium that only a few special cases have been solved. Most of these have been reduced by approximations to isotropic cases. The most important contributors have been Christoffel, Mason and Koga.

It seemed desirable to make as complete a study as possible of the type of vibrations occurring most frequently in quartz plates used for practical purposes, that is, the vibrations associated with the thickness dimensions.

THEORETICAL

The methods of tensor analysis were employed because they seemed so well adapted to the solution of the problem.

The tensor representing the stresses is denoted by the symbol, Φ_{ij}. This tensor is symmetric. The strains in the medium are defined by the equation

$$\Theta_{ab} = \frac{1}{2}\left(\frac{\partial u_a}{\partial x_b} + \frac{\partial u_b}{\partial x_a}\right)$$

where Θ_{ab} is the strain tensor, the u's are the displacements in the medium and the x's are the coordinates. This tensor is also symmetric. Hooke's relation between the stresses and strains is

$$\Phi_{ij} = c_{ijab}\Theta_{ab}$$

where the c's are the elastic constants of the medium. The c's possess symmetry such that a reversal of the first or last pair of indices or of the position of the two pairs does not change the value of the constants.

The equations of equilibrium and motion for elastic media are derived by the usual methods. One has

$$\mathbf{F} = \operatorname{div} \Phi \quad \text{or} \quad \rho\ddot{u}_j = \frac{\partial \Phi_{ij}}{\partial x_i}$$

where F is the force per unit volume of the medium.

By substituting the value of Φ from Hooke's relation into the latter equation the following equation is obtained:

$$\rho\ddot{u}_j = \frac{\partial}{\partial x_i} c_{ijab}\Theta_{ab}$$

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[1] Original thesis submitted July, 1936. Doctoral thesis number 395.

and when the value of Θ_{ab} in terms of the u's is introduced into this equation it becomes

$$\rho \ddot{u}_j = \frac{\partial}{\partial x_i} c_{ijab} \frac{\partial u_a}{\partial x_b}$$

when the symmetry properties of the constants are used.

The oscillator is assumed to be a plate whose x_1 dimension is d and whose other dimensions are infinite. This allows the assumption of the propagation of plane standing waves with harmonic time factors. The solution $u_j = A_j \cos \frac{n\pi x_1}{d} e^{i\omega t}$ is substituted into the differential equation. This gives the equations

$$-c_{1ja1} A_a \frac{n^2\pi^2}{d^2} + \rho\omega^2 A_j = 0$$

to be satisfied or if $\frac{\rho\omega^2 d^2}{n^2\pi^2}$ is set equal to k^2,

$$c_{1ja1} A_a - K^2 A_j = 0$$

These represent a set of three linear homogeneous equations in the three unknowns, the A's or amplitudes of vibration. They can hence have solutions only if the determinant of the coefficients has a value of zero. Setting this determinant equal to zero gives a third order equation in k^2. The solution of this equation gives three values of k^2. Substituting these back into the A equation gives linear relations between the A's.

$$A_1 = C_1 A_2 = C_2 A_3$$

for each value of k^2. These relations represent the normal modes of vibration corresponding to each value of k^2. No constant values are obtained for the A's indicating that the amplitude of vibration should not affect the frequency. The frequency is given by the equation

$$f = \frac{\omega}{2\pi} = \frac{nk}{2d\rho^{1/2}}$$

Since the coordinate axes chosen are related to the natural axes of the crystal only through the elastic constants it is recognized that the resonance frequencies corresponding to any orientation can be found if the x'_1 axis for this orientation is assumed to be normal to the surface of the plate and the elastic constants corresponding to that particular set of axes are found.

The elastic constants transform like a fourth order tensor, that is,

$$c'_{abcd} = l_{a\alpha} l_{b\beta} l_{c\gamma} l_{d\delta} c_{\alpha\beta\gamma\delta}$$

where the l's are the direction cosines between the axes involved. The fact that many of the constants for quartz are zero and the symmetry

properties of the constants simplifies the work of transformation considerably.

Because of the symmetry of quartz only one-twelfth of all the possible orientations needed to be investigated. A sufficient number of orientations to permit covering the entire range of possible orientations accurately by interpolation were chosen and the values of k calculated for them.

It is evident that since only finite plates could actually be used, that some difference can be expected between the theoretical and experimental frequencies. It should be expected that thin plates will, however, closely approximate the theoretical predictions. It may be expected that because of boundary effects frequencies not predicted by the infinite plate theory should appear. It may also be expected that the frequencies of the higher order harmonic modes should more closely approximate the corresponding theoretical frequencies than should the harmonic modes of lower order.

EXPERIMENTAL

All of the experimental plates used were finished so that the orientations were correct within 15′ and the surfaces were parallel within one-thousandth of a mm.

All measurements of frequencies were carried out by means of the filter method, that is, by using the quartz plate as a filter between an oscillator of variable frequency and a sensitive radio frequency voltage measuring device. Both frequency and amplitude of vibration of the plate were measured for each mode of vibration.

A test of the uniformity of quartz was first carried out. Four X-cut plates and four Y-cut plates were cut from the same crystal and finished to identical dimensions. Two X-cut and two Y-cut plates were also cut from different crystals and finished to the same dimensions. The modes of vibration of all the plates were found and the frequencies of the different plates checked against each other. It was found that both the frequencies and the amplitudes of vibration checked very closely in all cases. It was therefore concluded that a plate of a given orientation and given dimensions would give the same results no matter what crystal it was cut from.

Since it seemed impractical to check every orientation for which the values of K were calculated four orientations were chosen more or less at random. As each orientation possessed three modes of vibration this gave a total of twelve possible values of k which was thought sufficient to check the accuracy of the theory. Four plates were cut in the orientations chosen and their resonance frequencies measured. The third and fifth harmonics were measured whenever it was possible to find them. The experimental values of k thus found agree satisfactorily with the theoretical values. It is concluded that the infinite plate theory is a satisfactory approximation for thickness vibrations of a thin plate. It was observed as expected that the experimental harmonic frequencies agreed more closely with the corresponding theoretical harmonic frequencies as the order increased.

It was found in all cases that many resonant frequencies appeared which were not predicted by the infinite plate theory. They tended, how-

ever, to form groups near the frequencies predicted by the theory. Some of these were definitely shown to be associated with the lateral dimensions by experiments carried out which showed the change in the resonance frequencies as the lateral dimensions were changed.

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RELATIVE REACTIVITIES OF ORGANOALKALI COMPOUNDS[1]

RICHARD V. YOUNG

From the Department of Chemistry, Iowa State College

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A systematic study of the relative reactivities of the organoalkali compounds has not been reported in the literature. Because of their extreme reactivity there has been a tendency of workers in this field to place them apart as a group of very reactive compounds in which very little distinction has been made between their relative reactivities.

Organometallic compounds react at different rates with various groups, and by a judicious choice of the organometallic to be used for any synthetic purpose it may be possible to obtain selective reactions in which only certain groups of a polyfunctional molecule react to any marked extent. These selective types of reactions have not been applied to organoalkali compounds in general, but are found particularly in the chemistry of organomagnesium compounds which have been studied much more intensively. Of the greatest importance to the organic chemist, is the ability to predict, with reasonable accuracy, the course of reactions of this type, and a knowledge of relative reactivities of the organometallic compounds is indispensable in making these prognostications. Gilman and Nelson[2] have also demonstrated that by the choice of organometallic compounds of appropriate reactivity it is possible to greatly increase the yields of many of the products of the reaction.

A. RELATIVE REACTIVITIES OF 4-DIBENZOFURYLALKALI COMPOUNDS

The reaction velocities of 4-dibenzofuralpotassium and -sodium with fluorobenzene, chlorobenzene, and *o-tolunitrile* were studied. The procedure used during the experiments involved the addition of the reactant to a standardized diethyl ether solution of the 4-dibenzofurylalkali compound, and subsequently measuring the time required for the reaction to reach completion. The end-point of the reaction was determined by the color test method with Michler's ketone[3].

The results of the experiments are given in table 1. The numbers, with the exception of zero, indicate time in minutes. Zero indicates an almost instantaneous reaction.

B. RELATIVE REACTIVITIES OF TRIPHENYLMETHYLALKALI COMPOUNDS

The relative reaction velocities of triphenylmethyllithium and -sodium with chlorobenzene, fluorobenzene, and *o-tolunitrile* were determined. Standardized diethyl ether solutions of the triphenylmethylalkali compounds were allowed to react with a definite excessive amount of the reagent, and the time for the reaction to reach completion was determined

[1] Original thesis submitted July, 1936. Doctoral thesis number 396.

[2] Gilman and Nelson, *Rec. trav. chim.*, 55, 518 (1936).

[3] Gilman and Schultze, *J. Am. Chem. Soc.*, 47, 2002 (1925).

TABLE 1. *Reactions of 4-dibenzofurylalkali compounds with phenyl halides and o-tolunitrile*

	4-Dibenzofuryl-sodium	4-Dibenzofuryl-potassium
Fluorobenzene	20, 25.	15, 20, 20.
Chlorobenzene	25, 30, 30.	35, 40, 30.
o-Tolunitrile	3, 0.	0, 0.

by the disappearance of the characteristic color of the triphenylmethylalkali compound. The results of the experiments are given in table 2. The numbers indicate the reaction time in hours of the run and the check. A plus sign after the number indicates that the reaction was not complete in the given length of time.

TABLE 2. *Reactions of triphenylmethylalkali compounds with phenyl halides and o-tolunitrile*

	Triphenylmethyl-lithium	Triphenylmethyl-sodium
Chlorobenzene	14, 16, 17.	40+, 40+,
Bromobenzene	10, 8, 12.	40+, 40+.
o-Tolunitrile	0.04, 0.04.	0.02, 0.03.

C.. RELATIVE REACTIVITIES OF ALKYLALKALI COMPOUNDS

Equal molar quantities of ethyl-lithium, -sodium, and -potassium were allowed to react with a standard excessive amount of dibenzofuran in a petroleum ether solution. The reactions were terminated after two and one-half hours, by carbonating the reaction mixture with solid carbon dioxide. The extent of the reaction was determined by titrating the resulting dibenzofurancarboxylic acids with standard sodium hydroxide. The more reactive ethylalkali compound yielded the larger quantities of dibenzofurancarboxylic acids. The results of these experiments will be found in table 3. The numbers indicate the volume in cubic centimeters of standard sodium hydroxide required to neutralize the dibenzofurancarboxylic acids. The several values indicate check runs.

TABLE 3. *Titration of dibenzofuran acids with standard sodium hydroxide*

Ethyl-lithium	0.1	0.05	......
Ethylsodium	4.5	4.7	4.5
Ethylpotassium	14.1	12.6	15.0

D. RELATIVE REACTIVITIES OF PHENYLETHINYLALKALI COMPOUNDS

The relative reaction rates of the phenylethinylalkali compounds were determined by measuring the time required for the organoalkali compound to react with a definite excessive quantity of benzonitrile. Phenylethinylmagnesium bromide was included in these experiments so that a direct comparison of organomagnesium and organoalkali compounds could be made. Table 4 summarizes the results of the experiments. The values indicate time in hours required for the reaction to reach comple-

tion. The various values indicate check runs. Two different sets of values are given for phenylethinylpotassium. The first values are those that were obtained under conditions comparable to the organometallic compounds above them. The second values are those that were obtained under conditions similar to those of the compounds given below them.

TABLE 4. *Reactions of phenylethinylalkali compounds with benzonitrile*

Phenylethinylmagnesium bromide	87, 85.0
Phenylethinyl-lithium	57, 63.0
Pnenylethinlsodium	6.5, 7.0
Phenylethinylpotassium	4.3, 4.5
Phenylethinylpotassium	5.3, 5.1
Phenylethinylrubidium	4.8, 5.2
Phenylethinylcesium	3.7, 3.6

E. MISCELLANEOUS EXPERIMENTS

I. REACTIONS WITH SODIUM-POTASSIUM ALLOY

Sodium-potassium alloy was allowed to react with 2-phenyl-*iso*-propyl methyl ether, triphenylmethyl ethyl ether, diphenylmethyl methyl ether, triphenylmethyl-lithium, tetraphenylethylene, and triphenylmethane. In each case the organopotassium compound was formed, and no trace could be found of an organosodium compound. This is in accordance with the generalization that organopotassium compounds are more reactive than the analogous organosodium compounds[4].

II. CARBONATION OF ORGANOSODIUM COMPOUNDS

In contrast to organolithium and magnesium compounds[5], many organosodium compounds can be carbonated at room temperature to give excellent yields of the corresponding carboxylic acids. Phenylethinylsodium, -potassium, -rubidium, and -cesium; 4-dibenzofurylsodium; phenylsodium; tetraphenylethylenedisodium were carbonated at room temperature and yields from 60 to 78 per cent of the corresponding carboxylic acids were obtained.

[4] Gilman and Straley, *Rec. trav. chim.*, 55, (1936).

[5] Gilman and Van Ess, *J. Am. Chem. Soc.*, 55, 1258 (1936).

A REPORT OF SOME RECENT STUDIES ON SPECIES OF GASTEROPHILUS OCCURRING IN HORSES IN THE UNITED STATES

R. W. Wells and E. F. Knipling

From the United States Department of Agriculture, Bureau of Entomology and Plant Quarantine

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Although much has been published on the various species of Gasterophilus infesting the horse, certain phases of their life histories remain obscure. In the present paper the authors present some additions to the knowledge of the biology of the American forms, some elaborations on data previously published on certain phases, some facts to correct observations and interpretations reported in other publications, keys for identification of adults, and keys and figures for identification of the second- and third-instar larvae.

Investigators who are now delving more minutely into the larval habits of the several species, veterinarians who wish to be more precise in the evaluation of certain treatments, student veterinarians and student entomologists, all may use to advantage a single article giving keys for identification of the adults and of the second and third instars of the four species with which our horses are likely to be infested. In the preparation of the figures here given (Plates I and II), differentiation has been a main objective.

SPECIES CONCERNED

In the United States there are three well-established species of Gasterophilus. In the order of what appears to be their relative abundance in the Central States, these are *G. nasalis* L., the throat botfly; *G. intestinalis* DeGeer, the common botfly; and *G. haemorrhoidalis* L., the nose botfly. A fourth species, *G. inermis* Brauer, has been taken recently from a native horse, as reported by Knipling (8). This species may eventually become of great economic importance.

Of our three principal species, the adults, the eggs, and the first and third instars have been rather fully described and figured in a number of publications. Descriptions of the second instars of *Gasterophilus intestinalis* and *G. haemorrhoidalis* were given by Gedoelst (5). Dinulescu (2) described and figured the first instar of *G. inermis*.

KEY FOR THE IDENTIFICATION OF ADULTS OF GASTEROPHILUS FOUND IN THE UNITED STATES

(Adapted from Dove (4) and Dinulescu (2))

1. Wings hyaline, without cloudy patches.
   - a. Anterior basal cell equal or nearly equal in length to the discoidal cell .... *Gasterophilus nasalis* L.
   - b. Anterior basal cell markedly shorter than the discoidal cell .... *Gasterophilus haemorrhoidalis* L.

2. Wings with cloudy patches near the center and apex.
   a. Third trochanter with prominent spur..*Gasterophilus intestinalis* De Geer.
   b. Third trochanter without spur..........*Gasterophilus inermis* Brauer.

KEY FOR THE IDENTIFICATION OF THIRD-INSTAR LARVAE

1. Spines arranged in two rows, those of the first row more developed than those of the second row.. 2.
   Spines arranged in single row.................................*Gasterophilus nasalis.*
2. Spines tapering to a fine point; the band of spines on dorsum of segment 10 interrupted by at least half the width of the segment; segment 11 devoid of spines on dorsum... 3.
   Spines blunt at tip; only one to two pairs of spines lacking on dorsal center of segment 10; dorsum of segment 11 generally with from 1 to 5 spines above the lateral line on either side..*Gasterophilus intestinalis.*
3. Larva light reddish; ventral band of spines on segment 3 generally interrupted by a spineless area, but if not interrupted, the number of spines on venter never as numerous as on the venter of segment 4 ....*Gasterophilus haemorrhoidalis.*
   Larva light yellow; ventral band of spines on segment 3 not interrupted, the number of spines on this segment approximately the same as on segment 4...*Gasterophilus inermis.*

KEY FOR THE IDENTIFICATION OF SECOND-INSTAR LARVAE

1. Spines arranged in one band situated near the anterior margin of segments; the band made up of two to four irregular, closely approximated rows of alternating spines... 2.
   Spines arranged in two narrow, distinctly separated bands situated near the anterior margin of segments; the spines in the anterior band the larger and composed of two irregular, closely approximated rows of alternating spines; the more posterior band composed of two to three irregular, closely approximated rows of small spines..*Gasterophilus nasalis.*
2. Posterior spiracular slits (Pl. II, fig. 13) each with 18 to 22 transverse bars .... 3.
   Posterior spiracular slits with 12 to 13 transverse bars...*Gasterophilus inermis*[1].
3. Dorsal spines on corresponding segments greater in number (on segment 9, for example, the number of spines ranging from 34 to 48); band of spines on venter of segment 10 interrupted on median line...*Gasterophilus intestinalis.*
   Dorsal spines on corresponding segments fewer in number (on segment 9, for example, the number of spines ranging from 16 to

[1] This character, distinguishing *Gasterophilus inermis* from *G. haemorrhoidalis* and *G. intestinalis,* is based on the descriptions of *G. inermis* by Dinulescu (3).

33); band of spines on venter of segment 10 generally not interrupted...*Gasterophilus haemorrhoidalis.*

The first-instar larvae of *Gasterophilus nasalis, G. intestinalis,* and *G. haemorrhoidalis* have been adequately described by Hadwen and Cameron (6), Patton and Evans (10) and Dinulescu (2, 3). The author last mentioned also described and figured the first-instar larva of *G. inermis* (2).

The eggs of *Gasterophilus nasalis, G. intestinalis,* and *G. haemorrhoidalis* have been figured and described by Dove (4), Hadwen and Cameron (6), Bishop and Dove (1), Patton and Evans (10), and Dinulescu (3). Dinulescu has also figured and described the egg of *G. inermis* (2).

GASTEROPHILUS NASALIS L.

SEASONAL ACTIVITY

In the vicinity of Ames, Iowa, maturing larvae of *Gasterophilus nasalis* begin to leave the host in the early part of May. The earliest dropping recorded in 1933 was May 13 and in 1934 the earliest was May 12. Maturing larvae collected from slaughtered horses on August 26, 1933, pupated readily and emerged a few weeks later as adults. Adults were active as early as May 31 in 1934, and fresh eggs were found as early as June 7 in 1933.

At Galesburg, Ill., oviposition occurred as early as June 7 in 1932. Adults become less active during the hotter part of the summer, but activity increases again in the fall, though not to the extent of that in June. At Ames, Iowa, in 1933, oviposition occurred as late as October 29, and in 1934 as late as November 7.

MATING AND OVIPOSITION

This species seems reluctant to mate in captivity, only 2 pairs mating out of 40 pairs observed. They failed to mate while confined in a large screen cage with a horse. The writers have not observed this species to mate around horses in the open, as do *Gasterophilus intestinalis* and *G. haemorrhoidalis,* the males of both of which hover around the horses waiting for the appearance of females. No male of *G. nasalis* has ever been taken by the writers in the open.

The process of oviposition has been well described by Dove (4). The writers have found as many as five eggs on one hair, although usually only one. An undisturbed fly deposited 20 eggs without leaving the horse, though usually the reaction of the horse causes the fly to desist after one strike, and usually several minutes elapse before there is another approach, presumably by the same female. Eggs were counted in 8 reared females, the totals ranging from 304 to 515 and the average being 465. Dove (4) counted 480 to 518; Dinulescu (3) counted 480 to 510.

INCUBATION PERIOD AND HATCHING

The minimum incubation period has been found to be considerably shorter than as published in previous literature. Eggs deposited on the host on June 7 hatched in 5 days $\pm$ 2 to 17 hours. Eggs deposited on a horse on June 11 hatched in 4 days and 22 hours. In an incubator at

$91 \pm 1°$ F. the incubation period was 4 days and 14 to 20 hours. Patton and Evans (10) gave the incubation period as 9 to 12 days.

As is commonly known, the eggs of *Gasterophilus nasalis* are deposited on the hair investing the intermaxillary region from the chin to the pharynx, and, as has often been observed, the eggs hatch on the host apparently independent of external moisture and without any pressure or friction being applied by the host. The writers have found them to hatch at room temperature in covered, dry, paper pill boxes, as well as when placed on wire gauze exposed to natural and artificial light. At a favorable temperature, time for incubation appears to be the only needed factor.

LARVAL MOVEMENTS AND DEVELOPMENT

After emerging from the eggshells, the larvae of *Gasterophilus nasalis* are able to crawl readily on dry surfaces and are relatively resistant to desiccation. On the contrary, moisture seems essential to the welfare of both *G. intestinalis* and *G. hemorrhoidalis* immediately after they hatch, and indispensable to the hatching of the latter. Once hatched, the larvae of *G. nasalis* crawl and wriggle through the hair, close to the skin and always downward toward the lips, between which they make their way actively into the mouth.

The method by which the larvae of this species reached the stomach of the host was a speculative subject until Wells (12) observed this habit of crawling downward to the mouth. Since then the writers have repeated the observation several times and have conducted experiments which demonstrate the habit more conclusively. In one instance 25 newly hatched larvae were placed among the hair under the jaws of a horse approximately 8 inches higher than the commissures of the lips. Twenty minutes later one larva entered the mouth. Of the 25 larvae, 9 were observed to enter the mouth and 4 came within 1 to 2 inches of the mouth, then either fell to the ground or died among the hair. Generally the larvae proceed directly toward the mouth until they are approximately opposite the commissures of the lips, then change their course toward the lips. None were observed to change their downward course sooner. Observations were similar in several other tests made. A total of 20 larvae have been observed to enter the mouth. Of this number, 18 turned from their downward course when they had descended to a region approximately opposite the commissures of the lips and entered the lips near the commissures. The other 2 larvae continued their downward course and entered the mouth at the fore part of the lips.

That the response of these newly hatched larvae is geotropic is shown again by their action when placed among hair on another part of the horse. On the breast of the horse a margin around a rectangular patch about 4 inches square was closely clipped. Larvae attempting to cross this closely-clipped swath in the hair could be detected readily. Then 16 newly hatched larvae were placed with a fine brush among the hair in the center of the patch delineated by the clipped margin, and the marginal swaths were observed closely for migrating larvae. After 3 minutes the first larva began to cross the lower horizontal margin. Within 15 minutes 10 of the 16 larvae had been seen crossing the same margin. No larvae were found crossing any other part of the clipped margin; hence their habit is evi-

dently to crawl downward, and when the eggs have been placed along the jaw, this would bring them to the mouth of the horse.

Dinulescu (3) has stated that the larvae of *Gasterophilus nasalis*, upon entering the mouth, burrow into the mucosa of the cheeks, where they remain during the first instar. The writers' experiments have yielded no information on the habit of this species in the mouth of the horse. Careful examination of the buccal tissues of the horse made 1, 5, 6, 7, 8, 10, 11, 12, 13, 17, 19, 24, 30, and 38 days after newly hatched larvae were deliberately placed in the mouth did not reveal larvae in such tissues. In the duodenum, however, the larvae were recovered as second-instar larvae, in significant sizes. A horse, previously kept free from infestation, was deliberately infested with groups of newly hatched larvae, five times at weekly intervals for 5 weeks and was killed and examined on the 38th day after the first infestation. The second-instar larvae found in the duodenum, when grouped according to distinct differences in size, were apparently of three distinctly different ages, 24, 30, and 38 days, respectively, since these were the ages of the three older groups. The larvae next younger, but not found, were 17 days old. If it is a valid assumption that the larvae pass to the duodenum promptly after the first molt, then it may be concluded that the length of the first larval instar is between 17 and 24 days. The period was more closely defined when another horse, which had been kept free from natural infestation, was given a single lot of larvae in the mouth and killed 19 days later. Several small second-instar larvae were found in the duodenum, but no larvae were found in the tissues of the mouth. Thus the range of the developmental period of the first stage is narrowed to 17 to 19 days.

The durations of the second and third larval stadia have not been determined. As to the total larval developmental period, the deduction is that it is approximately 11 months, the larvae passing from the host as early as May 12 and the flies ovipositing as early as May 31.

Freshly dropped larvae of *Gasterophilus nasalis* are whitish in appearance and thus are easily distinguishable from those of the other common species, which are somewhat reddish. Moreover, larvae of *G. nasalis* are more sluggish, many being easily found in the manure several hours after dropping. In the pasture two were found to have pupated in the droppings. Larvae collected from the floor of the stable and placed on sand invariably gain cover beneath the surface.

PUPATION

The shortest prepupal period in the writers' records is 10.5 ± 1 hours. The average prepupal period during May and June at Ames, Iowa, was 18 hours. The pale yellow color persists until pupation is apparent, then changes to light red, to dark red, and to almost black, successively. The case hardens as the color darkens. The anterior spiracles, which are hidden beneath the larval cuticle, are forced out during the onset of pupation, and for the first hour or two of pupation their appearance is the only sure evidence of pupation.

The pupal periods at Ames, Iowa, based on exposures made from May 23 to June 19 in 1933, were found to range from a minimum of 16 days at room temperature to 20 and 21 days out of doors. At a constant temperature of 70° F. the period ranged from 32 to 36 days. Larvae taken from the

duodena of horses slaughtered on August 27 and 28 and placed, after pupating, an inch under soil out of doors, changed to adults 33 to 64 days after pupation began; whereas, pupae from larvae similarly obtained on September 11 and 12 failed to become adult. This seems a fairly dependable indication that larvae leaving the host later than August, at Ames, Iowa, are not likely to change to adults, nor, as in this instance, do the pupae survive the winter.

At other localities, and by other investigators, the pupal periods have been found to be quite different. Dove (4) recorded 25 to 56 days in South Dakota, and Dinulescu (3) reported 20 to 24 days at a temperature of 22° to 25° C. (71.6°-77° F.).

GASTEROPHILUS INTESTINALIS De Geer

Gasterophilus intestinalis, probably because of its conspicuous adult activity as well as the glaring abundance of its eggs on the coats of horses, has been popularly regarded as our most abundant species. Within horses dissected in Illinois and Iowa, however, the writers have found the larvae less abundant than those of *G. nasalis*.

SEASONAL ACTIVITY

Adult activity with *Gasterophilus intestinalis* begins a little later than with *G. nasalis*. At Galesburg, Illinois, June 24, 1931, and June 20, 1932, were the earliest dates on which activity was found; at Ames, Iowa, June 26 was the earliest in 1933. The activity, however, is occasional rather than common until August, when adult activity increases, and on through September, when, in Iowa and Illinois, the peak of activity occurs. The writers have found the flies active on the warmer days of October. They were active at Ames, Iowa, on October 5, 1933. Then came a period of 24 days with no activity, during which the minimum temperatures reached the low point of 21° F. on the night of October 24. Following this, from October 29 to November 1, were 4 days when maximum temperatures ranged to about 70° F., and adults were active on all days except possibly the last. In 1934 the flies were active throughout most of October, but not abundant; and November 7 was the last date of activity noted.

Adults have emerged from pupa cases when the air temperature was as low as 55° F. and were able to fly at 60° F., although they were not known to attempt oviposition at such a temperature. When the temperature dropped to 55° they were apparently unable to fly. On October 29, 1933, five flies emerged from pupa cases 1 inch under soil, out of doors, after a pupal period of 61 days and after the air temperature had dropped to 21° F. on the night of October 24. In the writers' judgment, November 1 can be taken as a date later than which, in latitudes similar to those of Ames, Iowa, adult activity of *Gasterophilus intestinalis* is negligible.

MATING AND OVIPOSITION

Mating by *Gasterophilus intestinalis* is generally begun during flight. The males hover about the forelegs of the horse, engage the females while in flight, then both fly a short distance and alight on the ground or on some object to complete copulation. They mate rather readily in captivity, having done so in glass vials 1 inch in diameter, in fruit jars, in screen cages 2 by 2 by 2.5 feet in size, and in a large screen cage occupied also by a

horse; they mate more readily, however, in the larger spaces where flight is possible. Unmated females in the large cage were not observed to attempt oviposition, but were seen flying about the legs of the horse apparently waiting for a mate. Only a few infertile eggs were deposited on the horse by such females. Females reared and mated in captivity oviposited readily on a horse confined in the large screened cage. Some females had mated and begun oviposition within an hour after emergence.

Oviposition occurs while in flight. In no instance have the writers seen the fly alight on the horse. Usually the eggs are attached along the distal half of the hair, and several eggs are often found on one hair. The eggs are found most thickly deposited on the inside of the front leg between the knee and the hoof; but eggs have been found on all parts of the front legs. The outside of the forearm, the region back of the elbow, the flanks, and the mane often carry many eggs. It is not, however, until in September and October that many eggs are found in the last three places mentioned.

One fly placed 301 eggs on a horse in 45 minutes; another fly placed 905 in the course of 2 hours and 45 minutes, and when dissected was found to have exhausted her supply. To determine the total egg capacity, counts were made, by dissection, on reared gravid females. The totals ranged from 795 to 935, the average in 7 females being 861. Dove (4) records a minimum of 397 and a maximum of 770, his average in 5 being 541. Dinulescu (3) counted from 1,006 to 1,046.

INCUBATION PERIOD

Eggs deposited on a horse July 7 were ready to hatch on the 5th day. Freshly deposited eggs removed from the host and kept at a temperature of 91 to 93° F. were fully incubated 76 hours later. As would be expected, incubation is greatly delayed by cool weather. Some eggs deposited on October 5 had not fully incubated on the host on October 20. In another instance eggs were deposited on two horses on October 29 and 30, and some were clipped off at regular intervals. On November 30 only one egg that was apparently fully developed was found; on December 20 approximately 40 per cent were not completely incubated; on December 28, or 59 to 60 days after oviposition, the last date of observation, 8 of 50 eggs examined were incompletely incubated. In another experiment eggs deposited on August 22 were clipped from the horse and placed in an electric refrigerator operating at 38° to 42° F. At intervals some were removed to room temperature and later found to be incubating. Finally, on December 5, after 105 days of such refrigeration, some of the eggs removed to room temperature achieved complete incubation, though the larvae were deformed and not very energetic.

ENDURANCE OF THE LARVA WITHIN THE EGGSHELL

When the larva of *Gasterophilus intestinalis* is fully developed within the eggshell, its first instinct is to gain entrance to the mouth of the horse. The opportunity is presented when the horse brings the lips into contact with the hairs on which the eggs are placed.

How long the larva may live within the eggshell, waiting for the casual contact from the horse's lips, has been a matter of much interest and economic significance. For, as is to be more fully shown, such larvae continue to invade the mouth of the horse in the fall of the year, long after the flies

have ceased to oviposit. A thorough riddance of bots in the stomach is only a temporary achievement unless these unhatched larvae still on the hair have been eliminated.

It was found that larvae within the eggshells died sooner at room temperatures than did larvae kept in a cooler place. Fresh eggs deposited on August 22 were kept at room temperature for 2 weeks, thus permitting complete incubation. Then approximately one-half of them were placed in a refrigerator operating at 38° to 42° F. Those remaining at room temperature were all dead within 60 to 79 days after deposition; of those under refrigeration, 2 per cent yielded living larvae 140 days after deposition.

A few living larvae were found in eggs taken from a horse near Ames, Iowa, on February 9, 1934, 100 days after November 1, the latest probable date of deposition.

In table 1 are presented data on the viability of eggs examined during the fall and winter periods of several years. It is shown that the percentage of living larvae continues high through October and November, and that the percentage is comparatively low after December 22.

TABLE 1. *The number of viable eggs of Gasterophilus intestinalis on various dates during fall and winter, 1932 to 1934*

Date eggs collected	Number of horses	Number of eggs examined	Number of eggs containing living larvae	Percentage of larvae alive
Oct. 21-23, 1933	12	3,137	1,535	48.9
Nov. 1-6, 1933	13	2,395	990	41.3
Nov. 21, 1933	4	492	165	33.5
Nov. 21-26, 1934	20	2,775	722	26.0
Nov. 26, 1932	5	1,126	312	27.7
Dec. 22-29, 1932	17	1,700	107	6.3
Jan. 2, 1933	13	1,300	54	4.2
Jan. 5, 1934	4	750	53	7.1
Jan. 17-18, 1933	22	2,200	86	3.9
Feb. 9, 1934	5	840	2	0.24
Feb. 15, 1933	10	950	0	0.0
Feb. 23, 1934*	4	1,092	0	0.0

* Columbia, Mo.; all others in the vicinity of Ames, Iowa.

HABITS OF THE FIRST-INSTAR LARVA

It has been well understood that the larvae of *Gasterophilus intestinalis* gain entrance to the host by way of the mouth, and that the larvae leave the eggshell promptly when the horse's lips are applied to the hair carrying the eggs. It has been demonstrated that the principal stimulus which prompts the larvae to hatch is a sudden rise to favorable temperature (9). The warm lips of the horse provide this sudden rise in temperature, the pressure and friction of the lips seem to aid convection of the heat, and the moisture from the lips helps to extricate the issuing larva from the eggshell and delays the hazard of desiccation.

Once inside the mouth the larvae soon burrow in the mucosa on the dorsal side of the anterior end of the tongue. This was shown by Dinulescu

(3), who used the guinea pig as a host. Wehr (11), using the horse as host, found that the larvae burrowed no deeper than the mucous membrane, and that they were found mostly in the stratum germinativum. In the mucosa they were found to burrow slowly toward the posterior end of the tongue, always on the dorsal side, in a course about parallel to the right or left lateral margin. The sinuous lines of the lesions, easily seen, are seldom near the median line of the tongue, but usually nearer the lateral margins. Dinulescu (3) gave sketches to show the trends of the lesions, and showed that along the burrows are found small openings and that usually the larvae were found with the posterior stigmata at one of these holes. The writers have found that the younger larvae make several such holes per inch of tunnel, but that the older or larger larvae make only about one hole to approximately 1 inch of tunnel.

To determine what percentage of the larvae hatching succeeded in establishing themselves in the mouth, approximately 905 eggs of *Gasterophilus intestinalis* were deposited on a horse which had been kept free from eggs. Seven days later all the eggs were clipped from the horse, and 205 were found to have hatched. On the following day the horse was killed and carefully examined, but only 14 larvae (6.8 per cent of those that hatched) were found in the tongue.

By the time the larvae reach the base of the tongue they have increased greatly in size and are near the first molt. Dinulescu (3) observed on some larvae the cuticulum of the second stage showing beneath the cuticulum of the first stage. Wehr (11) concluded that the maximum period in the tongue was 28 days. The writers consider that the probable minimum period in the tongue of the horse is 24 days. A horse was killed 24 days after 510 freshly hatched larvae had been placed in the mouth. Twenty-one first-instar larvae were found in the tongue, but none were found in the stomach. Apparently, then, the larvae occupy the tongue during a period of from 24 to 28 days.

It has not been definitely determined just when the first molt takes place. Dinulescu (3) stated that the larvae molt immediately after they come out of the tunnels at the base of the tongue. It seems more likely that the larvae abandon the old skin as they emerge, leaving the exuvium within the tunnel. Only second-instar larvae have been found attached to the pharynx and sides of the epiglottis. No one has shown either that the larvae pause very long in the pharynx or epiglottis or that they invariably pause and attach in those places.

Passing on to the stomach, then, as second-instar larvae, they do not undergo another molt until some time after 5 weeks. Necropsy was made on a horse 9 weeks after a group of newly hatched larvae had been placed in the mouth of the animal. Only second-instar larvae were found in the stomach. Deducting 4 weeks, as the maximum period established for the first instar, there remains the interval of 5 weeks during which the second molt had not occurred.

SEASONAL OCCURRENCE OF LARVAE IN THE TONGUE OF THE HORSE

Having discussed the incubation period, and the length of time the delitescent larvae of *Gasterophilus intestinalis* may remain in the tongue, the question now arises as to how late in the season eggs and larvae surviving on the coat of the horse are a source of infestation after oviposition

has ceased and after fumigation of the stomach. To determine this, tongues of horses from Story County and adjacent counties in Iowa were obtained from a local rendering plant. At intervals during the months of December and January in the winters of 1932-33 and 1934-35 the tongues of such horses were taken to the laboratory and minutely examined. Knipling (7) has shown the results obtained the first winter, and his data are included here in table 2, wherein is given a tabulation of the findings of the two seasons, which were in general similar. As the season advanced, the average number of larvae per tongue declined. After January 15 only a negligible number were found in the tongue. Since these had been in the tongue no longer than 28 days, it seems reasonable to conclude that very few larvae enter the host later than December 15.

TABLE 2. *Seasonal decline in number of Gasterophilus intestinalis in tongues of horses at Ames, Iowa*

	Winter of 1932-33			Winter of 1933-34		
Date tongues examined	Number of tongues examined	Range in number of larvae per tongue	Average number of larvae per tongue	Number of tongues examined	Range in number of larvae per tongue	Average number of larvae per tongue
Dec. 1- 7	5	0-20	10.4	16	0-46	9.25
8-14	8	5-63	26.0	17	0-22	4.76
15-21	5	0- 8	4.6	1	4	4.0
22-31	2	2- 5	3.5	15	0-20	3.27
Jan. 1- 7	9	0-14	3.2	10	0-5	1.70
8-14	9	0- 7	1.55	12	0-10	3.42
15-21	9	0- 1	0.11	18	0-5	0.55
22-27	....	....	....	16	0-1	0.13

DESTRUCTION OF EGGS OF GASTEROPHILUS INTESTINALIS

Gasterophilus intestinalis has been most active in depositing its eggs on the coat of the horse during August, September, and October. When fully developed within the eggshell these larvae, hereafter to be referred to as "latent" larvae, do not immediately emerge, but wait for the fortuitous contact of the horse's warm lips. Hence, by the first of November there is found on the coat of the horse the largest accumulation of eggs still unhatched, and at the same time a large procession of larvae burrowing in the tongue, but activity of the adults has practically ended. With an efficient prophylactic measure against the remaining eggs, a veterinarian may administer stomach treatment after an interval of 28 days with the assurance that the stomach will not receive immediate reinfestation from the coat and tongue.

Because of the fact that eggs of *Gasterophilus nasalis* are not accumulative, but have hatched independently and have passed to the stomach, no consideration need be given to their eggs. Eggs and buccal larvae of *G. haemorrhoidalis* are likewise not to be considered at this part of the season.

TABLE 3. *Effects of corncob oil on eggs of Gasterophilus intestinalis*

Type of oil	Host no.	Date eggs treated	Date eggs clipped	Date examined	Unhatched eggs examined				
					Number examined	Eggs not fully incubated		Latent larvae	
						Number	Percentage	No. dead	No. alive
Middle fraction	1	Nov. 24, 1933	Nov. 29, 1933	....	31	23	74.19	3	5
	2	" " "	" " "	Nov. 30, 1933	73	16	21.92	52	5
	3	Dec. 8, 1933	Dec. 11, 1933	Dec. 19, 1933	190	90	47.37	93	7
	4	" " "	" " "	" " "	56	2	3.57	35	19
	5	" " "	" " "	" " "	51	0	0.00	35	16
	6	" " "	" " "	" " "	186	74	39.78	110	2
	7	" " "	" " "	" " "	157	28	17.83	125	4
Twice distilled	8	Oct. 23, 1934	Oct. 24, 1934	Oct. 25, 1934	106	23	21.70	79	4
	9	" " "	" " "	Oct. 30, 1934	191	89	46.60	102	0
					1,041	345	33.14	634	62

Percentage surviving 5.96

WARM-WATER METHOD

The writers have published (9) their experiments on the destruction of the eggs of *Gasterophilus intestinalis* by applying warm water to the horse. The simplicity and efficacy of the method place it far ahead of any other method tried. To review briefly, it consists of sponging copiously the infested portions of the coat with water at 104° to 118° F. on a day when the air temperature is below 60° F. (15.6° C.). This treatment decoys the waiting larvae from the eggshell, after which they die from exposure without gaining entrance to the mouth of the horse. Experience with the method indicates that by such procedure the elimination of about 95 per cent of such latent larvae may confidently be expected.

CORNCOB OIL

The latent larvae were found to be invulnerable to chemical treatments tried on the coat of the horse with one exception. A tar oil distilled from the tars derived from the destructive distillation of cellulose waste is the most deadly substance, both to the fully developed, unhatched larva and to the eggs not completely incubated, that the writers have tried. Quantities of this oil were supplied by the Agricultural By-products Laboratory of the Bureau of Chemistry and Soils, United States Department of Agriculture. Mr. P. Burke Jacobs, in charge, called attention to the oil, which apparently had shown some value as a repellant in connection with some preliminary experimental work. Mr. Jacobs stated that these complex oils, derived from the distillation tars of various cellulosic materials, are essentially very similar. So far the oils have been a by-product. The oil used in the tests was an acid-free oil derived from the distillation tars of corncobs.

To test the value of this corncob oil on eggs of *Gasterophilus intestinalis* it was applied liberally with a cotton swab to the infested parts of the horse. The treated eggs were clipped from the horses 1, 3, and 5 days after treatment, then held at room temperature for from 1 to 8 days before final examination. Finally each egg was pricked open and the contents noted under a microscope. The results of these examinations are given in table 3.

The eggs from hosts Nos. 3 to 7 were held, after clipping, at room temperature for 7 days, a period found to be more than sufficient for completion of incubation. Hence it seems safe to conclude that none of the eggs found to be immature had survived the treatment, the only survivors being those in which the larvae were completely developed. Accordingly, the percentage of 5.96 considered as surviving is based on the total number of eggs examined and active larvae found.

The corncob oil used has about the same viscosity as water, but is not soluble in water. In no way has it seemed injurious to the horses, but being oily and dark colored it gives the animal's coat an unpleasant appearance, especially when used on horses of lighter color. It also persists for several days and collects dust, and the pungent, tarry odor is exceedingly difficult to remove from the hands and clothing of the operator, a consideration of importance to those handling fancy animals, and to farmers who are obliged to milk cows or handle dairy products. Where for any reason, however, the use of the oil may seem more expedient than the warm-water method, already described, it can be depended on to give

TABLE 4. *Effects of phenol washes on unhatched larvae of Gasterophilus intestinalis*

Treatment	Host No.	Number of unhatched eggs examined	Number of larvae: Dead	Number of larvae: Alive	Percentage surviving
Phenol 2 per cent	60	100	2	98	98
	17	100	4	96	96
	11	100	1	99	99
	78	100	0	100	100
	36	100	3	97	97
	45	100	3	97	97
	85	100	0	100	100
	28	100	0	100	100
	53	100	0	100	100
	35	100	2	98	98
		1,000	15	985	98.5
Phenol 3 per cent	31	100	2	98	98
	38	100	0	100	100
	27	100	2	98	98
	67	100	5	95	95
	64	50	0	50	100
		450	9	441	98.0
Phenol 4 per cent	68	100	15	85	85
	40	100	15	85	85
	22	100	10	90	90
	76	39	10	29	74.4
	41	100	9	91	91
	55	100	5	95	95
		539	64	475	88.13
Cresol (U.S.P.) 2 per cent	49	100	1	99	99
	7	100	2	98	98
	44	100	2	98	98
	34	100	1	99	99
	12	50	0	50	100
		450	6	444	98.67
Cresol (U.S.P.) 3 per cent	70	100	1	99	99
	65	100	1	99	99
	39	100	1	99	99
	21	57	1	56	98.2
		357	4	353	98.88

satisfactory results. The oil is not yet available on the market, but a large potential supply exists in the by-products of the hardwood-distillation industry, and an evident demand would undoubtedly be met by the provision of adequate quantities of the oil of approximate standardization.

OTHER CHEMICALS

In general, the writers have found that after complete development within the eggshell, the larva is remarkably invulnerable to nearly all medications tried that are known to be tolerated by the skin of the horse.

In groups of 50, the eggs, and hairs to which they were attached, were immersed for 1 minute in a small amount of the solution in an evaporating dish; then they were lifted out of the solution with forceps and placed on towel paper saturated with the same solution. The saturated towel papers bearing the eggs were laid into small baskets of copper screen to provide full ventilation. The baskets containing the eggs were then placed in constant temperature at 30° C. (86° F.) where they remained until the fourth day. The operculum of each egg was then lifted and the contents of the eggs observed under the microscope. Eggs not containing active larvae were considered dead. Untreated eggs of both ages, for checks, were held at 30° C., and all of them contained active larvae on the same day the treated eggs were examined.

Eggs fully incubated (4 to 7 days old) as well as eggs only slightly incubated (3 to 12 hours old) did not survive immersion in the laboratory for 1 minute in a 2 per cent aqueous solution of phenol, but when such a solution is applied to the coat of the horse, only the partly incubated eggs are vulnerable, as will be shown presently. Of eggs of the same aged groups immersed in a 2 per cent aqueous solution of liquor cresolis for the same length of time, 77.8 per cent of the well incubated eggs survived, but the immature embryos were all killed. Likewise, a 1 per cent solution of pyrethrum in kerosene-mineral oil was fatal only to the unincubated eggs. Of these 94 per cent were killed, while none of the well incubated eggs succumbed.

That the latent larvae (larvae waiting within eggshell after incubation is complete) are largely invulnerable to phenol and cresol in concentrations tolerable to the skin of the horse seems supported further by the results of bathing the infested coats of 30 horses. (Table 4.)

On September 18 representative samples of eggs were clipped from 10 of the horses, and 97.95 per cent of the unhatched eggs contained active larvae. On September 19, 9 to 10 A. M., the 30 horses were washed on the infested parts with the solutions, applied liberally with a piece of cheesecloth and rubbed sufficiently to drench the skin. Twenty-four hours later liberal samples were opened under the microscope and the condition of the latent larvae noted. Eggs not fully incubated were disregarded, since the object of the test was to determine the effect on latent larvae.

Constant movement of the vessels containing the solutions and the frequent agitation in immersing the cloth obviated the possibility that the solution was not uniform in concentration. It was found that 4 per cent phenol and 3 per cent cresol were very irritating to the skin of the hand. From the results of this test it may be concluded that phenol washes are not deadly to the latent larvae and therefore cannot be recommended as ovicides.

GASTEROPHILUS HAEMORRHOIDALIS L.

SEASONAL ACTIVITY OF ADULTS

The earliest observance of the adults of *Gasterophilus haemorrhoidalis* at Ames, Iowa, in 1933 was on June 8; in 1934 they were active at Fort

Des Moines, Iowa, on June 1. Activity in the fall of the year at Ames has not been observed. Dove (4) recorded the adults active as late as October 10 in South Dakota. At Ames, Iowa, larvae were observed to leave the host as early as May 7 in 1934, and as early as May 9 in 1935. Cavalrymen at Ames, Iowa, reported the larvae dropping on May 1 in 1933.

MATING AND OVIPOSITION

Gasterophilus haemorrhoidalis mated readily in captivity. It oviposted readily on a horse confined in a screened corral, but not as readily as does *G. intestinalis*. Like *G. intestinalis*, the males linger in the vicinity of the host waiting for the appearance of the females. Males reared and released have been observed to linger around a horse for 6 hours. The male darts for the female while the latter is still in flight and the pair settle 10 to 50 feet away from the horse, on the ground or some object, and complete copulation, which takes from 5 to 10 minutes. Females were seen oviposting immediately after copulation. On one occasion 3 male and 2 female laboratory-reared flies that were released were under observation for 3 hours, around a lone horse closely tethered, and during that period 20 to 30 copulations were observed among that group of flies.

The preoviposition period has been as short as 55 minutes. In this instance the female emerged at 8:20 A. M., mated in captivity at 8:40 A. M., and deposited a fertile egg on the horse by 9:15. In several instances ovipositions occurred within 2 hours after emergence.

Dove (4) states that the black eggs are placed by the female on the short hairs fringing the lips of the horse. The pedicel of the egg is snugly attached to the base of a hair. Counts of the eggs placed by different females at different times on the lips of 3 horses, showed that 90 out of 112 were on the upper lip. Most of them were found about 1 inch from the hair line of the lips, though some were found along the hair line. A few were three-fourths of an inch below the nostrils.

Records made by the writers showed females carrying 51 to 208 eggs. Counts from 8 reared females yielded an average of 160.3 eggs.

INCUBATION PERIOD

The writers have found incubation to be complete in about 2 days, either on the host or when incubated artificially. Flies were permitted to deposit eggs on the lips of two horses tied within a screened cage. The horses had not been exposed previously and were found to carry no eggs. From 9 A. M. to 3 P. M., 15 eggs were deposited on the lips of the two horses on June 9. At 9 A. M., June 13, or 3 days and 21 $\pm$ 3 hours after the deposition, only 3 eggs were found, and these had hatched. Again, on June 15, between 8:20 A. M. and noon, two eggs were deposited on a horse. At 10:30 on June 18, or 3 days $\pm$ 1½ hours later, these two eggs had hatched. A more valuable observation was made on June 18, when 17 eggs were deposited on a horse between 1 P. M. and 7 P. M. Observation was made at 1 P. M., June 20, when the 8 eggs recovered were examined under the microscope. Four of the 8 had hatched and 4 contained fully formed and active larvae. Hence the incubation period may be as short as 45 $\pm$ 3 hours. Eggs collected within 1.5 hours after deposition and kept in pill boxes at room temperature hatched when placed in water 46 hours $\pm$ 45 minutes after deposition.

FACTORS AFFECTING THE HATCHING OF GASTEROPHILUS HAEMORRHOIDALIS

From observations and experiments it appears that after sufficient incubation, and with favorable temperature, the only other essential requirement for hatching is moisture.

Dove (4) stated that the eggs of *Gasterophilus haemorrhoidalis* hatch in the presence of moisture and friction. The present writers find that, in favorable temperature, moisture is the only essential stimulus. From a room temperature of 30° to 32° C. (86°-89°F.), fully incubated eggs, in duplicate lots of 5 each, were placed in water heated to 24°, 27°, 40°, and 45° C. (75.2°, 80.6°, 104°, and 113° F.), respectively. The percentage of hatch was about the same at each temperature. Of the 10 in water at 24° C. all hatched except one. Friction is therefore not essential, and in the presence of free water, a change of temperature is not necessary to hatching. Next it was found that there was no hatching response to a rise in temperature in the absence of free water. Five eggs held for several hours in an air temperature of 4.5° to 7° C. (40.1° to 44.6° F.) were abruptly transferred to an air temperature of 45° C. (113° F.). None of the five hatched in the course of 4 minutes. These eggs were then placed in water at 35° C. (95° F.) and all hatched within 1 to 3 minutes. Another lot of 5 eggs with the same initial exposure (4.5° to 7° C.) were quickly transferred to 50° C. (122° F.) air temperature. None hatched in 4 minutes. Next they were placed in water at 35° C., but none hatched. The eggs were opened and the larvae were dead, apparently killed by the exposure to 50° C.

Only one egg among over a hundred eggs handled hatched in the absence of free water. Hadwen and Cameron (6), however, reported 8 hatching among 14 in a dry vial.

The egress of the larva of this species from the eggshell is relatively slow, 30 seconds being the minimum time recorded. Generally it requires 2 to 3 minutes after moisture has been applied; rarely, 9 minutes has elapsed before the exit is complete. This response was slow compared to the rapid egress of *Gasterophilus intestinalis* from the egg. *G. intestinalis* bursts open the operculum as if by one thrust, whereas *G. haemorrhoidalis* lifts the cap by successive efforts, a little at a time. The former has to seize the brief opportunity to gain the horse's lips, whereas the latter, already on the lips, is at the point of penetration into the tissues of the host, as shall be shown presently.

ENDURANCE OF THE LARVAE WITHIN THE EGGSHELL

Eggs of *Gasterophilus haemorrhoidalis* removed from the host immediately after deposition and placed at room temperature hatch readily on the fourth day, but do not survive beyond the sixth day. Ten eggs deposited on June 22 were placed in a pill box at room temperature. On the fourth day 5 were placed in water and all yielded active larvae. On the fifth day the remaining 5 were placed in water. Three of these hatched; the other two contained dead larvae. The test was repeated using 32 eggs. Of 5 placed in water on the fourth day, all hatched. No observations were made on the fifth day. On the sixth day all of the remaining 27 eggs were placed in water, but none hatched. Then 10 were opened and found to contain dead larvae.

HABITS OF NEWLY HATCHED LARVAE OF GASTEROPHILUS HAEMORRHOIDALIS

It has been a general assumption that the newly hatched larvae of *Gasterophilus haemorrhoidalis* either crawled into the mouth or were swept into the mouth by food and water. The authors have found that the larvae penetrate the epidermis of the lips and migrate in this tissue into the mouth. Within the mouth they continued for a while, at least, their subepithelial existence. Dinulescu (3) placed larvae of this species into the mouth of a guinea pig and found them to burrow into the mucous membrane of the tongue, as do the larvae of *G. intestinalis*. The writers have not found them in the tongues of horses naturally infested, but the larvae first invade the skin of the lips where they emerge from the eggshell. On June 22, and again on June 26, groups of reared flies of this species were released near a tied horse to be used as experimental host. The flies responded by depositing about 80 eggs on the first date. The second group deposited an equal number on the second date. On June 30 the horse was killed and examined. The tongue, the insides of the cheeks, and both the inner and outer surfaces of the lips were carefully examined. Larvae were found only in the lips. Many were found burrowing in the skin of both the upper and the lower lip, most of them just inward from the hair line. Others were embedded under the hair near the hair line. The lips were then cleanly shaven. No larvae were found embedded among the follicles on the lower lip. Among the follicles on the upper lip two larvae were found embedded, both headed toward the mouth; one was three-fourths inch and the other, one and seven-eighths inches from the edge of the mucous membrane. There were a number of sinuous lesions extending to the mucous membrane. Allowing 2 days for incubation, the eggs presumably hatched 6 and 2 days, respectively, before the postmortem. No doubt most of the larvae had reached the inside of the mouth in that period. The larvae were of two distinct sizes, presumably of the two different ages.

In order to observe the performance of a newly hatched larva two eggs were placed on the hairy portion of the lower lip, in position under the microscope, 4 hours after the death of the horse. Each egg was then moistened with a drop of water. In due time the larvae emerged from the eggshells and at once began to burrow at that place, and within a few minutes had disappeared beneath the epidermis. On another occasion 3 larvae of this species, distinctly larger than those 6 days old, were found burrowing in the lips of a horse. None were found in other parts of the mouth. It seems a fair conclusion that the larvae remained in the lips more than 6 days, and their burrowing in the lips must cause great discomfort to the horse.

As has been commonly observed, the second-instar larvae are found in the stomach, where they undergo a molt and continue their existence for a time before migrating for reattachment in the rectum. In horses at Ames, Iowa, second-instar larvae were found in the stomach as late as March 1. Of 100 third-instar larvae of this species taken from horses in Illinois on May 21 and 22, 65 were from the stomach and duodenum and 35 were from the rectum. It appears that the larvae do not migrate to the rectum before the middle of December. No larvae were found in the rectums of 40 horses examined in Illinois on December 11, 1933, whereas, 12 of the horses had an average of 3.4 larvae each in the stomach and duodenum; neither were any larvae found in the rectums of 13 horses ex-

amined in Iowa in December. These findings are of practical significance, for they indicate that the larvae of this species are in the stomach and duodenum throughout the winter, where they are subject to fumigation by the usual treatment.

PERPUPAL AND PUPAL PERIODS

Some of the larvae of *Gasterophilus haemorrhoidalis* plucked from the anus of a horse have pupated within 7.5 hours. Many have pupated within 12 hours. The majority pupated within 24 hours. The pupal period at room temperature has been as short as 15 days. Generally it is 1 day shorter than that of *G. nasalis* and from 2 to 3 days shorter than that of *G. intestinalis* when all 3 species of pupae are in the same environment.

COMPARATIVE ABUNDANCE OF GASTEROPHILUS SPP. WITHIN THE HOST

It appears that in the Middle West *Gasterophilus nasalis* is the most abundant within the host, *G. intestinalis* is second, and *G. haemorrhoidalis* is third. From January to March, 1932, determinations of the bots were made from the stomach and duodena of 12 horses at Ames, Iowa. Of the 2,767 bots, 1,593 were of *G. nasalis,* 1,147 were of *G. intestinalis,* and 27 were of *G. haemorrhoidalis.* Twenty horses from Des Moines, Iowa, were examined similarly on June 22, 1933, and of 2,350 larvae taken 1,360 were of *G. nasalis* and 990 were of *G. intestinalis.* On June 22, 1933, 40 horses from Dakota, Montana, and Illinois were examined and found to yield 8.815 larvae, of which 4,720 were of *G. nasalis,* 3,800 were of *G. intestinalis,* and 295 were of *G. haemorrhoidalis.*

While most of the counts given in the foregoing paragraph were made after maturing larvae had begun to pass from the host, it should be remembered that *Gasterophilus nasalis* begins to pass earlier in the season than *G. intestinalis.*

SPECIAL METHODS

Methods were developed to facilitate the collection of eggs of *Gasterophilus nasalis* and *G. haemorrhoidalis.* The contour of the region and the nervousness of the horse made it a difficult matter to clip a quantity of unhatched eggs of *G. nasalis* from under the jaw where the eggs are deposited. Moreover, it was essential to have eggs of known age. This problem was adequately solved by placing an egg trap along the jaw of the horse. The device consisted merely of a piece of horsehide of a dark color, tanned with the fur on. It was cut sufficiently large to cover the region of the jaw from the chin to the pharynx, and was laced securely to the straps of the halter, with the hair side out. Two halters with such strips were prepared for each horse, so that each day the infested halter could be replaced by the duplicate, and the one removed shorn of the eggs. Thus it was possible to secure each day eggs of limited age.

Finding such hair so useful for obtaining eggs of *Gasterophilus nasalis* the writers sought to adapt the method for obtaining eggs of *G. hemorrhoidalis,* which are attached to the hair on the lips of the horse. A piece of horsehide was cut and stitched in the shape of a bag, hair outside, large enough to place over the muzzle of the horse, holes being cut over each nostril to allow for breathing. Because of the dark color of the eggs of this

species, white hair made the eggs easier to find. It was found advisable also to clip the hair fairly short along the region of the lips, to simulate the short hair on the lips. The flies would deposit their eggs very readily on such hair, and the collection of the eggs of known age was greatly simplified.

It was found that immature larvae of *Gasterophilus haemorrhoidalis* and of *G. intestinalis* collected from slaughtered horses can be successfully transplanted into a living horse. The larvae were packed in gelatin capsules and passed into the esophagus with a balling gun. The larvae mature readily in the new host, as proved by the recovery later of marked larvae, apparently normally and fully developed. This proved to be a convenient way to obtain a large yield of larvae from one horse. *G. nasalis,* however, did not survive the transfer, as was indicated by the large number of dead larvae passing with the feces.

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EXPLANATION OF PLATES

PLATE I

Fig. 1. *Gasterophilus nasalis* Linn. Lateral view of egg. x 72

Fig. 2. *Gasterophilus intestinalis* DeGeer. Lateral view of egg. x 72

Fig. 3. *Gasterophilus haemorrhoidalis* Fall. Lateral view of egg. x 72

Fig. 4. *Gasterophilus inermis* Brauer. Outline drawing. Lateral view of egg. After Dinulescu.

Fig. 5. *Gasterophilus nasalis*. Newly hatched first instar larva. Ventral view. x 150

Fig. 6. *Gasterophilus intestinalis*. Newly hatched first instar larva. Dorsal view. x 140

Fig. 7. *Gasterophilus haemorrhoidalis*. Newly hatched first instar larva. Dorsal view. x 160

Fig. 8. *Gasterophilus inermis*. First instar larva. Dorsal view. Approximately x 150. After Dinulescu.

PLATE I

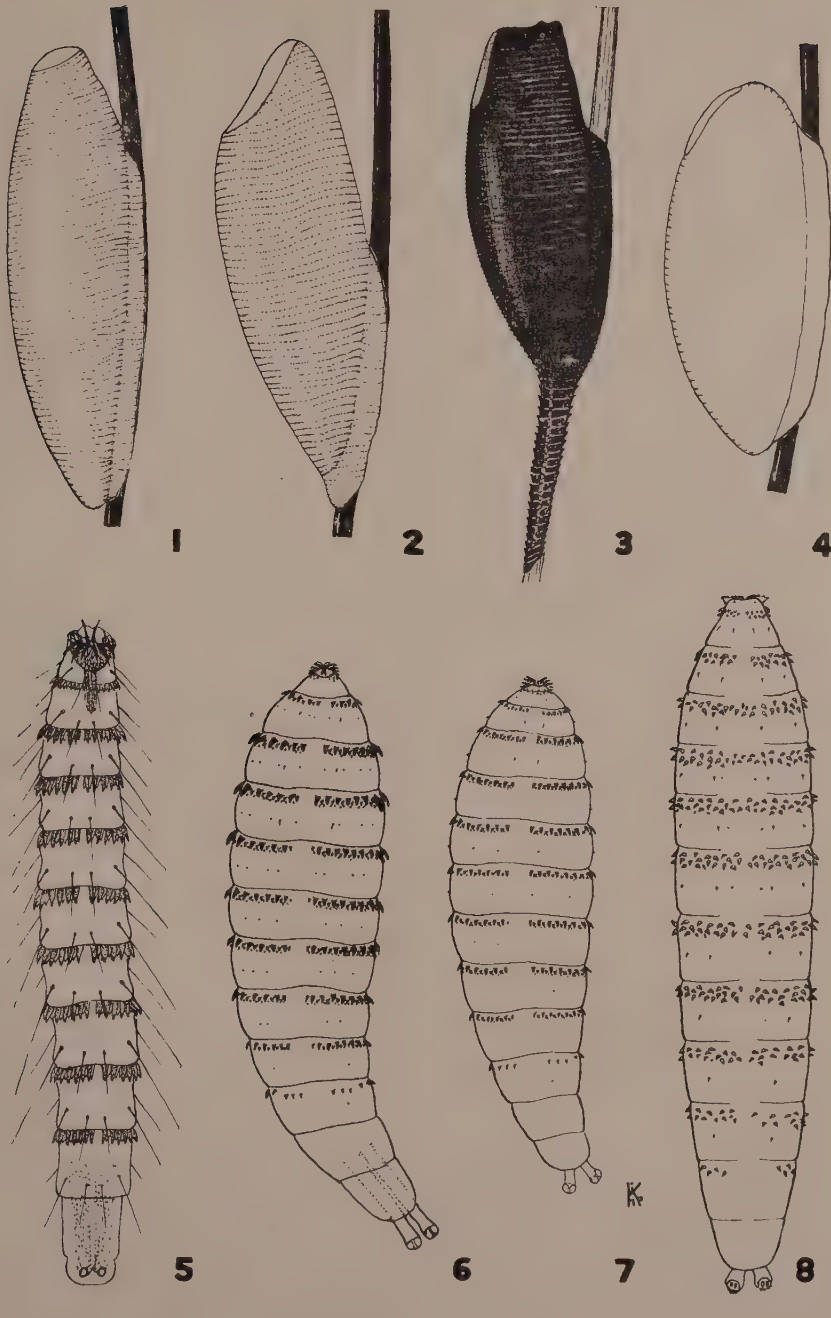

PLATE II

Fig. 9. *Gasterophilus nasalis.* 2nd instar larva. Dorsal view. x 14.

Fig. 10. *Gasterophilus intestinalis.* 2nd instar larva. Dorsal view. x 14.

Fig. 11. *Gasterophilus haemorrhoidalis.* 2nd instar larva. Dorsal view. x 14.

Fig. 12. *Gasterophilus nasalis.* Posterior Spiracles. 2nd instar larva. Approximately x 65

Fig. 13. *Gasterophilus intestinalis.* Posterior Spiracles. 2nd instar larva. Approximately x 65

Fig. 14. *Gasterophilus nasalis.* 3rd instar larva. Dorsal view. x 7.

Fig. 15. *Gasterophilus intestinalis.* 3rd instar larva. Dorsal view. x 7.

Fig. 16. *Gasterophilus haemorrhoidalis.* 3rd instar larva. Dorsal view. x 7.

Fig. 17. *Gasterophilus inermis.* 3rd instar larva. Dorsal view. x 7.

PLATE II

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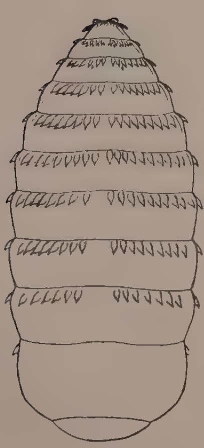

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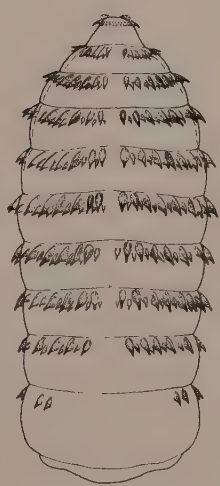

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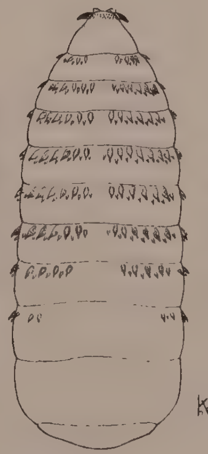

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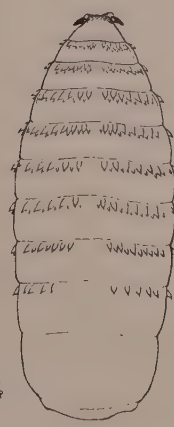

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FRACTIONATION OF OAT HULL LIGNIN[1]

Wilbur S. Claus and Ralph M. Hixon

From the Department of Chemistry, Iowa State College

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The preparation of ammonia lignin from oat hulls and the oxidation of this lignin with alkaline iodine solutions has been previously reported (1). This study has been continued in an attempt to fractionate, or purify, the iodo-carboxy-lignin and thus to eliminate the indefinite cannotation still necessary with the term lignin.

The fractionation was first attempted by repeated methylation and saponification of the iodo-carboxy-lignin. The methoxyl content of the saponified product increased as the reaction was repeated, a result analogous to the change reported for cornstalk lignin (2).

As a result of these observations, the original product prepared by Walde was investigated after storage for two years in a closed container. It was found that this methylated oxidized lignin ($OCH_3 = 22.2$ per cent), which at the time of preparation went completely into solution in hot 10 per cent sodium hydroxide to give Walde a soluble lignin with a methoxyl content of 17.4 per cent, would not go completely into solution under any set of circumstances[2]. This observation would indicate that the change is not solely due to the action of the alkali.

Similar changes have been observed in the ammonia lignin. Walde (1) reported that the freshly isolated ammonia lignin softened at 60° C. and that this characteristic property was lost after a few weeks. A partial reversal of this decrease in solubility can be affected by refluxing in 5 per cent sodium carbonate solution with the subsequent precipitation of the lignin from the carbonate solution (2). The available evidence is not sufficient to indicate whether these changes are structural or merely a change in the degree of molecular aggregation. The partial reversal of solubility by dilute carbonate solution and the fact that the changes take place upon storage in closed containers would favor the conclusion that these changes are primarily physical.

EXPERIMENTAL PROCEDURE

PREPARATION OF AMMONIA LIGNIN

The ammonia lignin was prepared from oat hulls according to previously described procedure (1). The analysis of the isolated product differed less than 2 per cent from ammonia lignin used by Walde.

PREPARATION OF THE OXIDIZED LIGNINS

The iodo-carboxy-lignin obtained from the acid-hydrolyzed oat hulls (abbreviated I. C. L. No. I) and the oxidized lignin obtained from the

[1] Supported in part by a grant from the Industrial Science Research funds of the Iowa State College for the study of lignin.

[2] This observation was substantiated by A. W. Walde.

ammonia lignin (I. C. L. No. II) were prepared according to the previous procedure (1) except that a 4 per cent sodium hydroxide solution was used instead of the previously reported 25 per cent sodium hydroxide solution.

The I. C. L. No. I was slightly soluble in dioxane, ethyl alcohol, isopropyl alcohol; and partially soluble in methyl alcohol and acetone. The I. C. L. No. II was completely soluble in 10 per cent sodium hydroxide, 5 per cent sodium carbonate, and 5 per cent sodium bicarbonate solutions; partially soluble in dioxane and acetone.

FRACTIONATION AND ALKALINE OXIDATION OF THE AMMONIA LIGNIN

The ammonia lignin was exhaustively extracted with acetone for 9 hours in a Soxhlet until the extractant liquor was colorless. The acetone soluble fraction was then concentrated by vacuum distillation to one-half its original volume and allowed to evaporate to dryness. The gummy residue was dissolved in ammonium hydroxide by slowly heating to 40°C. and then obtained as a light brown flocculent precipitate by the addition of concentrated hydrochloric acid until the solution was slightly acid to litmus. The lignin was filtered, redissolved, and reprecipitated in the above manner. On addition of the acid to the ammoniacal solution heated to higher temperatures, the lignin coalesced and became a heavy, black gummy mass. A 40 per cent yield of the acetone soluble fraction and a 55 per cent yield of the acetone insoluble fraction were obtained. A comparison of the composition of the two fractions is shown in table 1.

The alkaline iodide oxidation values were determined on the acetone soluble and the acetone insoluble fractions of the ammonia lignin using various conditions. The value found for the acetone soluble fraction was 211-212 cc. N/10 iodine per gram of lignin quite independent of the conditions. With the insoluble fraction, a preliminary period of heating the lignin with the caustic solution before adding the iodine caused an increase in the oxidation value from 184 cc. to 200 cc.

The sodium hypobromite oxidation values on the ammonia lignin were obtained in a manner similar to the values obtained by the sodium hypoiodite procedure. The procedure was as follows: To a weighed lignin sample in a 250 cc. iodine flask was added 10 cc. of water followed by 25 cc. of 1 N sodium hydroxide; the mixture was shaken till the lignin was completely peptized. To the alkaline lignin solution was added 50 cc. of a sodium hypobromite solution which had been prepared according to the procedure of Kolthoff (3). After the drop-wise addition of the alkaline hypobromite solution, the flask was stoppered and allowed to stand for 10 minutes. About two grams of potassium iodide was added to the solution followed by 25 cc. of 2 N sulfuric acid.. The liberated iodine was immediately titrated with standard sodium thiosulfate using starch as an indicator. A blank was also run using the same procedure. The excess thiosulfate used in the lignin titration was subtracted from the value for the blank to give the thiosulfate used in oxidizing the weighed lignin sample.

Oxidation of the acetone soluble fraction of the ammonia lignin with sodium hypobromite at room temperature gave a consistent oxidation value of 240 cc. N/10 bromine per gram of lignin. This figure was in close agreement with the value of 242 cc. on the non-fractionated ammonia

lignin. The sodium hypobromite oxidation values on the acetone insoluble fraction of the ammonia lignin were somewhat lower, but gave a consistent oxidation value of 226 cc. N/10 bromine per gram of lignin. Preliminary heating at various temperatures followed by the sodium hypobromite oxidation in the described manner did not change the oxidation values.

FRACTIONATION OF IODO-CARBOXY-LIGNIN

An attempt was made to fractionate the oxidized lignin by means of acetone as described above for the ammonia lignin. The oxidized lignin condensed with the acetone to give about a 10 per cent increase in weight. The greater portion (90 per cent) of the product was insoluble in acetone. The reactivity of the compound with the oxidizing agents was decreased as a result of the condensation of the lignin with acetone.

REPEATED OXIDATION OF IODO-CARBOXY-LIGNIN

It has been recognized that the extent of oxidation of lignin varied with the concentration of the reactants and with the previous treatment of the lignin. An effort was made to drive the reaction to completion by isolating the oxidized product and subjecting it to repeated oxidation. The quantity of oxidizing reagent used decreased from about 154 cc. of N/10 iodine per gram lignin in the first re-oxidization to 44 cc. in the second re-oxidation and practically zero in the third repetition. Iodoform was the only product isolated other then the iodo-carboxy-lignin. The percentage of recovery was approximately 80 per cent of the starting material. A comparison of the compositions of the re-oxidized lignins is shown in table 1.

METHYLATION OF OXIDIZED LIGNINS

Two methods of methylation were used upon the oxidized lignins. The procedure with diazomethane was to allow the sample to stand in an ether solution of diazomethane at room temperature for 18 hours. The solvent was decanted and the product washed twice with petroleum ether. The procedure with dimethyl sulfate was essentially that of Urban (4). All methylated products were dried in a vacuum oven over phosphorus pentoxide at elevated temperatures before analysis. Methylations were repeated until the methylated products showed no change in methoxyl content. Typical analytical results are shown in table 1.

DISCUSSION OF RESULTS

Wright and Hibbert (5) have pointed out the unreliability of methylating with dimethyl sulfate and caustic. Later Compton and Hibbert (6) substantiated this view and suggested as the best methylating conditions for avoiding structural changes: (a) the use of acetone as a solvent; (b) a slight excess of alkali (5-10 per cent); and (c) a temperature of 20° C. The authors state, however, that caution is necessary in drawing conclusions regarding structure from methylating studies using dimethyl sulfate, and caustic.

It is evident that acetone could not be used as a solvent in methylating the oxidized lignins since it condenses with them to give a 10 per cent increase in weight. The method of Urban (4) had been previously used in this laboratory, since it seemed to be the mildest procedure using dimethyl

sulfate. Referring to table 1, it is apparent that this method of methylation gave the same total methoxyl content as methylation with diazomethane for the acetone insoluble fraction of ammonia lignin and for the re-oxidized fractions of the iodo-carboxy-lignin. Whether the differences in methoxyl content between the two methods for the other products are caused by the structural changes discussed by Hibbert, or to inherent differences in the hydroxyl groupings of the parent products, must remain conjectural at the present time. It should be emphasized that as the oxidation reaction is driven to completion, the difference between the two methods of methylation disappears.

Numerous analyses which are not tabulated tend to show that the more soluble fractions of lignin have lower methoxyl values than their less soluble analogs. If it is assumed that the methylation with dimethyl sulfate and caustic with the described precautions give products which are completely methylated, then it can also be shown that the more soluble fractions have higher hydroxyl values than their insoluble analogs. These results are in accord with the findings of Wright and Hibbert (5).

TABLE 1. *Comparison of the percentage composition of various lignin perparations and their methylated derivatives*

Product	Percentage composition of parent lignin				Methylated by diazomethane		Methylated by dimethyl sulfate	
	C	H	OCH_3	I	OCH_3	I	OCH_3	I
A Acetone soluble ammonia lignin	61.6	7.1	10.6		23.3		29.0	
B Acetone insoluble ammonia lignin	63.8	6.5	11.1		22.8		23.0	
C Iodo-carboxy-lignin No. I	44.3	4.3	9.6	11.4	19.2	9.3	23.0	9.3
D Re-oxidized C	48.7	4.7	8.5	14.8	19.8	11.6	20.6	11.0
E Re-oxidized D	47.5	3.9	7.5	15.7	20.4	14.3	21.9	13.8

SUMMARY

Ammonia lignin prepared from oat hulls can be separated into two fractions according to solubility in acetone. This solvent condenses with the iodo-carboxy-lignin.

As the alkaline iodine oxidation is carried to completion, the structure of the lignin molecule is so modified that methylation with diazomethane and dimethyl sulfate give approximately the same methoxyl content.

The acetone insoluble fraction of ammonia lignin methylates to the same extent with diazomethane as with dimethyl sulfate.

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BIOLOGICAL ASSAY OF FEEDING STUFFS IN A BASAL RATION FOR COCCIDIUM-GROWTH-PROMOTING SUBSTANCE

II. BARLEY, RYE, WHEAT BRAN, WHEAT FLOUR MIDDLINGS, SOY BEAN MEAL[1]

ELERY R. BECKER AND RUSSEL C. DERBYSHIRE

From the Department of Zoology and Entomology, Iowa State College

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In a recent paper the writers (1937) described a method for the biological assay of certain food materials for the hypothetical (that is, as yet unidentified) substance that promotes the development of *Eimeria nieschulzi* in the white rat. Since the plan of procedure and treatment of data were outlined in the previous paper, it is unnecessary to repeat them here. In the present report the W-value represents the ratio of the mean growth gain by the animals on the test diet to the same by the animals on the control or reference diet during the first 16 days the diets were fed. The F-value, as before, is the ratio of the mean oöcyst counts for the animals on the test diet to the same for the animals on the reference or control diet.

ASSAY OF WHOLE BARLEY

Barley of the Velvet variety was obtained from a nearby farm and ground to a meal in a coffee mill. It was fed in the basal ration at the 30 per cent level. Barley, according to Morrison's (1936) tables, contains about 11.8 per cent protein, is a good source of vitamins B and E, contains appreciable amounts of vitamin G, and from none to appreciable amounts of vitamin A. The data recorded in table 1 show that in one trial the rats grew better on the barley mixture than on the control ration and in the second trial not so well. The W-value of .92 would indicate that there was very little difference between the two series so far as growth was concerned. The *F-value* of .98 would indicate the same for oöcyst production. It appears then that barley has about the same coccidium-stimulating property as wheat which, as stated in the previous report, gave an F-value of .92.

ASSAY OF WHOLE RYE

Rye of the Dakold variety was obtained from an Iowa farm and ground to a meal in a coffee mill. It also was fed in the basal ration at the 30 per cent level. Morrison's tables show that rye contains 12.3 per cent protein, is a good source of vitamins B and E, and has appreciable amounts of vitamin G.

Table 2 shows for rye a W-value of 1.5 and an F-value of 1.83. These are to be compared with a W-value of 1.63 and an F-value of .92 for whole wheat. The growth gains for rye and wheat were about the same, but

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rye furnished a far greater amount of coccidium-growth stimulant than wheat.

ASSAY OF WHEAT BRAN

Wheat bran was purchased from a local dealer. On account of its coarseness it was run repeatedly through a coffee mill with the burrs closely set in order to reduce it to a degree of fineness that would prevent selection by the rats. It was mixed in the basal ration at the 30 per cent level. The resulting mixture was quite bulky on account of the lightness of bran, but the rats ate it well. Morrison's tables show that wheat bran contains about 15.8 per cent protein and appreciable amounts of vitamin G, and is a good source of vitamins B and E.

As indicated in table 3, a *W*-value of 1.73 and an *F*-value of 1.91 were obtained for wheat bran. These values are to be compared, respectively, with 1.63 and .92 for ground whole wheat. While the growths of the rats on the two diets were about the same, the bran mixture favored the elimination of about twice as many oöcysts as the wheat.

ASSAY OF WHEAT FLOUR MIDDLINGS

The middlings were purchased from a local feed dealer, and mixed with the basal ration at the 30 per cent level. According to Morrison's tables, wheat middlings contain about 17.1 per cent protein, are an excellent source of vitamins B and E, and contain appreciable amounts of vitamin G.

Table 4 shows that a *W*-value of 1.72 and an *F*-value of 2.65 were obtained. The variability in ratio of weight gains in the test series to the

TABLE 1. *Oöcyst counts and weight gains for rats on 4 per cent powdered yeast and 30 per cent ground barley diets*

Trial	Rat number	(1) Reference series		(2) Test series		Ratio (2): (1)	
		Weight gain (gm.)	Oöcysts 10^6	Weight gain (gm.)	Oöcysts 10^6	Weight gains	Oöcysts counts
1	1	48	190	48	122		
	2	54	290	44	169		
	3	58	152	53	170		
	4	46	99	61	248		
	5	62	168	74	85		
	6	42	172	38	109		
	7	28	117	47	64		
	Mean	48.29	169.71	52.14	138.14	1.08	.81
2	8	61	125	21	240		
	9	60	173	34	225		
	10	32	221	36	194		
	11	34	187	38	239		
	12	59	177	43	180		
	Mean	49.2	176.6	34.4	215.6	.70	1.22
W						.92	
F							.98

TABLE 2. *Oöcyst counts and weight gains for rats on a 4 per cent powdered yeast and 30 per cent ground rye diets*

Rat number	(1) Reference series		(2) Test series		Ratio (2): (1)	
	Weight gain (gm.)	Oöcysts 10^6	Weight gain (gm.)	Oöcysts 10^6	Weight gains	Oöcysts counts
1	41	150	65	271		
2	36	151	53	279		
3	37	139	69	206		
4	46	76	44	241		
5	25	117	61	162		
6	34	73	45	223		
7	35	127	41	338		
8	41	149	69	139		
9	38	78	55	217		
10	40	156	57	148		
Mean	37.3	121.6	55.9	222.4	1.5	1.83
W					1.5	
F						1.83

TABLE 3. *Oöcyst counts and weight gains for rats on 4 per cent powdered yeast and 30 per cent wheat bran diets*

Trial	Rat number	(1) Reference series		(2) Test series		Ratio (2): (1)	
		Weight gain (gm.)	Oöcysts 10^6	Weight gain (gm.)	Oöcysts 10^6	Weight gains	Oöcysts counts
1	1	46	37	80	295		
	2	26	20	77	43		
	3	31	40	64	26		
	4	28	41	59	29		
	5	35	24	42	45		
	6	20	77	51	37		
	7	10	55	74	68		
	8	53	30	51	164		
	9	51	116	75	28		
	Mean	33.33	48.89	63.67	81.67	1.91	1.67
2	10	30	105	56	170		
	11	27	68	26	174		
	12	24	104	58	322		
	13	32	126	45	342		
	14	44	160	39	379		
	15	33	248	69	310		
	16	30	121	54	295		
	17	51	115	66	289		
	Mean	33.88	130.87	51.63	285.12	1.52	2.18
W						1.73	
F							1.91

same in the reference series is somewhat disconcerting, but it is to be noted that the one most out-of-line is trial 4. In this case the test series made unusually high gains and the reference series unusually low. The F-value of 2.65 is the highest yet obtained for any feeding stuff tested. Trial 3 was the most out-of-line with a ratio of 4.94. Such variability is difficult to explain on the basis of probability, and one must recognize the possibility of differences in the vitality of cultures of oöcysts. Since in all trials the ratios were high, it is evident that there is considerably more coccidium-growth-stimulating substance in wheat middlings than in whole wheat which, as previously stated, gave on the test an F-value of .92.

ASSAY OF SOY BEAN MEAL

The particular soy bean meal used in the experiment was obtained from a local dealer who purchased it from the Rath Packing Company, Waterloo, Iowa. It was represented as having been made by the process of expressing beans cooked at a high temperature. It had the characteristic "nutty" taste of high-temperature meal as distinguished from the "beany" taste of low-temperature meal.

Morrison's tables submit for expeller process soy bean meal about

TABLE 4. *Oöcyst counts and weight gains for rats on 4 per cent powdered yeast and 30 per cent wheat flour middlings diets*

Trial	Rat number	(1) Reference series		(2) Test series		Ratio (2): (1)	
		Weight gain (gm.)	Oöcysts 10^6	Weight gain (gm.)	Oöcysts 10^6	Weight gains	Oöcysts counts
1	1	46	69	79	382		
	2	49	111	72	132		
	3	52	105	64	196		
	4	33	82	51	211		
	5	....	....	48	156		
	Mean	45	91.75	62.8	215.4	1.40	2.35
2	6	45	78	67	147		
	7	59	74	51	103		
	8	46	95	50	151		
	9	54	82	50	128		
	Mean	51	82.25	54.5	132.25	1.07	1.61
3	10	52	96	54	330		
	11	54	67	65	295		
	12	46	55	60	282		
	13	52	19	57	265		
	Mean	51	59.25	59	293	1.16	4.94
4	14	28	26	72	24		
	15	17	62	47	70		
	16	30	27	63	108		
	17	5	41	78	64		
	Mean	20	39	65	66.5	3.25	1.71
W						1.72	
F							2.65

TABLE 5. *Oöcyst counts and weight gains for rats on 4 per cent powdered yeast and 10 per cent soy bean meal diets*

Trial	Rat number	(1) Reference series		(2) Test series		Ratio (2): (1)	
		Weight gain (gm.)	Oöcysts 10^6	Weight gain (gm.)	Oöcysts 10^6	Weight gains	Oöcysts counts
1	1	36	142	48	77		
	2	35	113	44	51		
	3	39	127	40	16		
	4	26	129	45	78		
	5	32	123	32	113		
	Mean	33.6	126.8	43.8	67	1.30	.53
2	6	50	112	40	70		
	7	42	92	38	49		
	8	57	114	47	17		
	9	50	116	43	51		
	10	55	83	46	53		
	Mean	50.8	103.4	42.8	48	.84	.46
3	11	36	181	36	176		
	12	49	242	38	106		
	13	44	207	23	86		
	14	40	127	37	83		
	15	42	124	32	25		
	16	32	118	49	48		
	Mean	40.5	166.5	35.8	87.33	.88	.53
W						1.00	
F							.51

44.3 per cent protein, and rate it as a good source of vitamin B. It contains also appreciable amounts of vitamin G.

On account of the high protein content of soy bean meal, it was used in the basal ration at the 10 per cent level.

The *W*-value of 1.00 indicates that over the 16-day growth period the test and control rations supported the same mean amount of growth. It is of further interest, however, that weights taken at the end of the sixth day following the 16-day period show the growth of the rats on the soy bean diet was much more seriously affected than of the series on the control diet. It was probably because of the scanty amounts of vitamins B and G in the soy bean meal, as well as the moderately light coccidial infection. The *F*-value of .51 is to be compared with .31 for meat and bone meal and .94 for linseed meal—other feeding stuffs fed at the 10 per cent level in the previous experiment by the writers (1937).

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THE DEVELOPMENT OF VASCULAR TISSUES AND THE INITIATION OF THE INFLORESCENCE IN HOLCUS SORGHUM

Chao-hsi Chen[1]

From the Department of Botany, Iowa State College

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The comparative anatomy of the embryo and seedling in the Gramineae has long been a fertile field for investigation. In recent years it has become evident that a knowledge of developmental anatomy is essential in the pursuit of agronomic and pathological studies, and emphasis is now directed toward complete developmental studies of selected species. Notwithstanding the importance of sorghum as a crop plant, the literature on the structure and development of this plant is meager. Other grasses have been studied much more intensively. Critical reviews of studies on the comparative morphology of the grass embryo and seedling have been presented by McCall (5), and Boyd and Avery (3).

The embryo and seedling of sorghum have been studied by a number of investigators, primarily from the standpoint of the homologies of the embryonic organs, as related to the comparative morphology of the Gramineous embryo. Boyd (2) and Reznik (6) have described the vasculation and histology of the embryo, and Sargant and Arber (7) have described the anatomy and morphology of the seedling. There is little available information concerning the origin and development of the complex and diverse tissues in the vascular bundle of sorghum. Similarities in stem organization of sorghum and corn (Hershey, 4) have suggested a similar course of histogenesis.

The present study was undertaken to determine the origin, sequence of differentiation and cellular structure of tissues of the stem. The approximate time of inception of the inflorescence during the growing season was also determined.

MATERIALS AND METHODS

The Kaoliang variety of Sorghum was used in this study. Greenhouse grown seedlings were used for the study of seedlings. Field grown plants were used for the study of later stages of vascular differentiation and of the initiation of inflorescence. Collections were made at weekly intervals for eight weeks after planting. The following killing solution was used:

1 per cent Chromic acid	20 cc.
1 per cent Acetic acid	75 cc.
37 per cent Formaldehyde	5 cc.

This solution kills and hardens the tissues in 48 hours. Embryos were prepared for imbedding in paraffin by removing the embryo intact from

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soaked kernels. Imbedded material was prepared for sectioning by soaking the imbedded pieces, mounted and oriented on hardwood blocks, in warm water for at least twelve hours. Sections were stained in hemalum and safranin, or safranin and fast green.

The drawings of low magnification were made with a calibrated microprojector. Detail drawings were made with a camera lucida or by micrometer measurements.

OBSERVATIONS

THE EMBRYO

The sorghum embryo, isolated from the caryopsis, presents in its longitudinal section, a well differentiated structure resembling the embryo of corn. The upper part of the axis, enclosed by the coleoptile, consists of the meristematic stem tip, and an average of five nodes bearing leaf primordia. The lower part of the axis, enclosed by the coleorhiza, consists of the meristematic radicle or primary root, with its root cap. Between the coleoptile node and the scutellar plate, there is an interval which is commonly designated as the "mesocotyl." Neither seminal roots nor axillary buds were found in the embryos of the variety investigated. Primordia of roots are evident in the mesocotyl one week after germination. The inception of root formation is probably much earlier. Sorghum does not have an epiblast.

VASCULAR TISSUES OF STEM

The differentiation of the vascular elements of the stem from the apical meristem is initiated by the formation of provascular strands. These strands can be recognized about two hundred microns from the tip of the stem of the embryo at the beginning of germination. (Fig. 1.) A strand is a cylindrical column of narrow, elongated cells which have thin walls, dense cytoplasm and relatively large nuclei. (Fig. 3.) When first recognizable, a strand is three to four cells in thickness. Rapid cell division in the longitudinal plane brings about an increase in the diameter of the strand. All cells of the strand may participate in this meristematic activity. At any given level, the cells and their nuclei are usually in transverse alignment, presenting a stratified appearance. Elongation of the group of cells at a given level takes place at a uniform rate, preserving the stratified arrangement until differentiation of tracheary elements begins.

In the provascular strands of the seedling stem, the cells soon begin their differentiation into xylem and phloem. The protoxylem and protophloem arise simultaneously. The first protoxylem element develops on the inner side of the strand. (Fig. 4.) This element is invariably an annular tracheal tube, having a typical thin primary wall and thick, lignified annular secondary thickenings. The length and diameter of the mature vessel segments, and the spacing of the rings, vary considerably. In longitudinal sections, all stages in the differentiation of this first protoxylem element can be traced. The process parallels the differentiation of similar elements as known to occur in other grasses.

The differentiation of xylem progresses centrifugally. During the later stages in the development of the first annular trachea, the cells of a second column begin to enlarge and differentiate (Figs. 5-6). These cells may form either an annular trachea, or a tube in which both annular and

spiral thickenings occur. There may be a radial series of three or more tracheal tubes exhibiting a progressive transition from annular to spiral or spiral-reticulate trachea.

Metaxylem elements can be recognized during the later stages of the differentiation of protoxylem. In transverse sections, a cell on each side of the latest protoxylem begins to undergo enlargement (Fig. 6). The nucleus, a thin, cytoplasmic layer, and numerous crossing strands of cytoplasm persist until maximum cell enlargement is attained. These two cells are part of vertical columns of cells which develop into pitted or reticulate-pitted trachea (Fig. 7). In typical elements, the thick, lignified secondary wall is perforated by oblique pits arranged in spiral order. The size of the pits varies in different trachea. Large oblique pits give the effect of reticulate secondary walls.

The vertical column of cells between the two large pitted metaxylem tracheal tubes develops into a strand of pitted tracheids (Figs. 7-8). These tracheids are short, prismatic cells with oblique end walls. The side walls and end walls are sparsely pitted. As a rule, the tracheids attain their structural maturity after that of the pitted trachea. In some bundles the walls of the metaxylem trachea remain unlignified while the pitted tracheids have already developed strongly lignified walls. In such cases, the pitted tracheids attain structural maturity earlier than the pitted trachea.

Protophloem elements, consisting of a group of three to five cells, can be recognized simultaneously with the earliest protoxylem cells. The young protophloem cells are not much larger than the adjacent provascular cells, but the former have relatively thick walls (Fig. 4). These walls have very little affinity for stains and present a translucent, glistening appearance. When the phloem cells are approximately ten in number, the sieve tubes have enlarged enough to be distinguishable from companion cells. The strand of phloem increases in diameter rapidly by division and enlargement of the cells. Differentiation of metaphloem takes place centripetally. The protophloem is gradually pushed outward and crushed by the expansion of metaphloem (Figs. 6-7).

The bundle sheath of sclerenchyma arises from the proliferated marginal cells of the developing bundle (Fig. 8). There are two massive areas of sclerenchyma, one at the xylem and one at the phloem side of the bundle, joined by a layer of sclerenchyma, usually one cell thick, extending around the radial sides of the bundle. The typical bundle sheath cell is a long, prismatic cell with thick, lignified secondary walls and a small cylindrical lumen. The ends are oblique, rather than tapered. Pitting is extremely reduced or entirely absent. There is comparatively little twisting or interweaving of cells.

The vascular bundles in the central region of the stem complete their differentiation earlier than the peripheral bundles. New provascular strands arise in the periphery of the stem after the protoxylem and protophloem of the central bundles are well developed.

Cells of the ground meristem continue to divide after the bundles of the same region have reached maturity. It is difficult to determine the exact time of termination of meristematic activity in ground meristem. The transition from ground meristem to mature pith parenchyma is progressive and continuous.

In the internodes, the peripheral bundles are crowded and smaller than those in the central region. The outermost circle of bundles forms

practically a solid ring, their confluent sclerenchymatous sheaths, together with the thickened epidermis and one to three layers of lignified hypodermis, constituting the tough "rind" of the culm.

INITIATION OF THE INFLORESCENCE

Young sorghum plants were collected at weekly intervals beginning with the first indications of germination. One week after germination the seedling has four or five well-developed foliage leaves, in addition to several leaf primordia newly laid down from the meristematic tip. This process of leaf formation continues until floral primordia arise. The first flower primordia become evident on approximately the thirty-fifth day and may be recognized by the appearance of small regularly-spaced lobes on the elongated terminal portion of the axis (Plate II, fig. 1). At this time, fourteen to eighteen leaves or nodes are present, representing the full number of the leaves or nodes of the mature plant.

After the third week bud primordia appear in the axils of three or four leaves immediately below the stem tip (Fig. 2). Axillary buds are well differentiated at the end of the fourth week. In the mature plant, a bud is present at each node or the axil of each leaf except the upper three or four nodes. Buds are older and more advanced structurally at the basal nodes.

DISCUSSION

Comparison of the structure of the sorghum embryo and seedling with corn and the more common cereal grasses reveals many similarities and some fundamental differences. In the absence of seminal roots, also reported by Reznik (6), sorghum differs from corn, wheat, oats and many other grasses. Axillary buds are present in the embryo of wheat and oats, whereas, corn and sorghum are similar in the absence of pronounced axillary buds, and in the comparatively late inception of such buds, approximately three weeks after germination.

The sequence in which axillary buds arise, and the degree of differentiation of the buds on a plant, have not yet been determined, but preliminary observations indicate that the upper three or four nodes, as in corn, lack discernible buds.

The differentiation of the vascular system of sorghum parallels that of corn (Hershey (4)) in the sequence of cellular development in the bundle, the order of differentiation of bundles in a stem, and in the gross aspects of nodal anatomy. Non-vascular tissues of the stem also exhibit a similar history in sorghum and corn.

The primordia of the terminal inflorescence of sorghum are laid down in approximately five weeks, the same interval necessary for the initiation of the staminate inflorescence of corn. A comparison of the developmental history of the terminal and axillary primordia of sorghum with those of corn would be of much interest, contrasting a plant with terminal fruiting with one normally having axillary fruiting. Further study of the details of development of the inflorescence and axillary buds of sorghum is to be undertaken. A comparison of varietal differences in rate and sequence of development is also proposed.

SUMMARY

1. A study was made of the differentiation of the vascular tissues of sorghum, *Holcus sorghum* variety Kaoliang. The time of initiation of the inflorescence was determined.
2. At the beginning of germination, the embryo has, on the average, five leaf primordia. No seminal root primordia occur in the embryo.
3. Provascular (procambium) strands of the stem differentiate from the apical meristem. Each strand consists of a cylinder of elongated, deeply staining cells. The strand increases in diameter by cell division and exhibits transverse stratification of cells.
4. In the provascular strands of the seedling stem, protoxylem and protophloem arise simultaneously. The first protoxylem element, an annular trachea, develops on the inner side of the strand. A group of three to five protophloem elements can be recognized on the outer side of the strand.
5. Differentiation of xylem is centrifugal, producing in succession, annular, spiral and reticulate protoxylem elements, pitted metaxylem trachea, and finally pitted tracheids.
6. Metaphloem differentiates centripetally and consists of sieve tubes and companion cells. The protophloem is pushed outward and crushed.
7. Provascular cells in the central and peripheral regions of the strand continue to divide during the differentiation of protoxylem and protophloem. Cells of the ground meristem also continue to divide until the bundles are almost completely differentiated.
8. The bundle sheath of sclerenchyma arises from the marginal cells of the developing bundle.
9. Vascular bundles in the central region of the stem complete their differentiation earlier than the peripheral bundles. New provascular strands arise in the periphery after the xylem and phloem of the central bundles are developed.
10. Axillary buds arise about four weeks after germination, first becoming evident at the third or fourth node below the stem tip.
11. Initiation of the inflorescence, terminally on the axis, occurs approximately five weeks after germination.

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PLATE I

Fig. 1. Apical meristem, leaf primordia and provascular strands in seedling of *Holcus sorghum* one week after germination. x 30.

Fig. 2. Axillary buds well-defined in plant four weeks after germination. x 30.

Fig. 3. Cross section of provascular strand near apical meristem. No indications of differentiation are evident. x 300.

Fig. 4. Strand showing first recognizable protoxylem and protophloem elements. x 200.
A. First distinguishable protophloem cell.
B. Protoxylem trachea most commonly annular.

Fig. 5. First protoxylem elements lignified, later protoxylem and metaxylem elements are nucleate, and in process of enlargement. Companion cells are evident. x 150.
C. Trachea, spiral or reticulate-spiral.
B. Protoxylem trachea most commonly annular.

Fig. 6. Protoxylem elements completely lignified, first-formed trachea ruptured to form lacuna. Metaxylem fully enlarged, but still nucleate. Protophloem becoming crushed. x 150.
C. Trachea, spiral or reticulate-spiral.
B. Protoxylem trachea most commonly annular.

Fig. 7. Trachea fully lignified, tracheids still unlignified. x 230.

Fig. 8. Mature bundle with all trachea, tracheids and bundle sheath sclerenchyma lignified, Xylem parenchyma evident. x 200.
G. Crushed protophloem.
F. Metaphloem companion cell.
E. Metaphloem sieve tube.
D. Metaxylem trachea, pitted.
H. Pitted tracheids.
C. Trachea, spiral or reticulate-spiral.
I. Xylem parenchyma.
J. Bundle sheaths, sclerenchyma.
K. Pith, parenchyma.

PLATE I

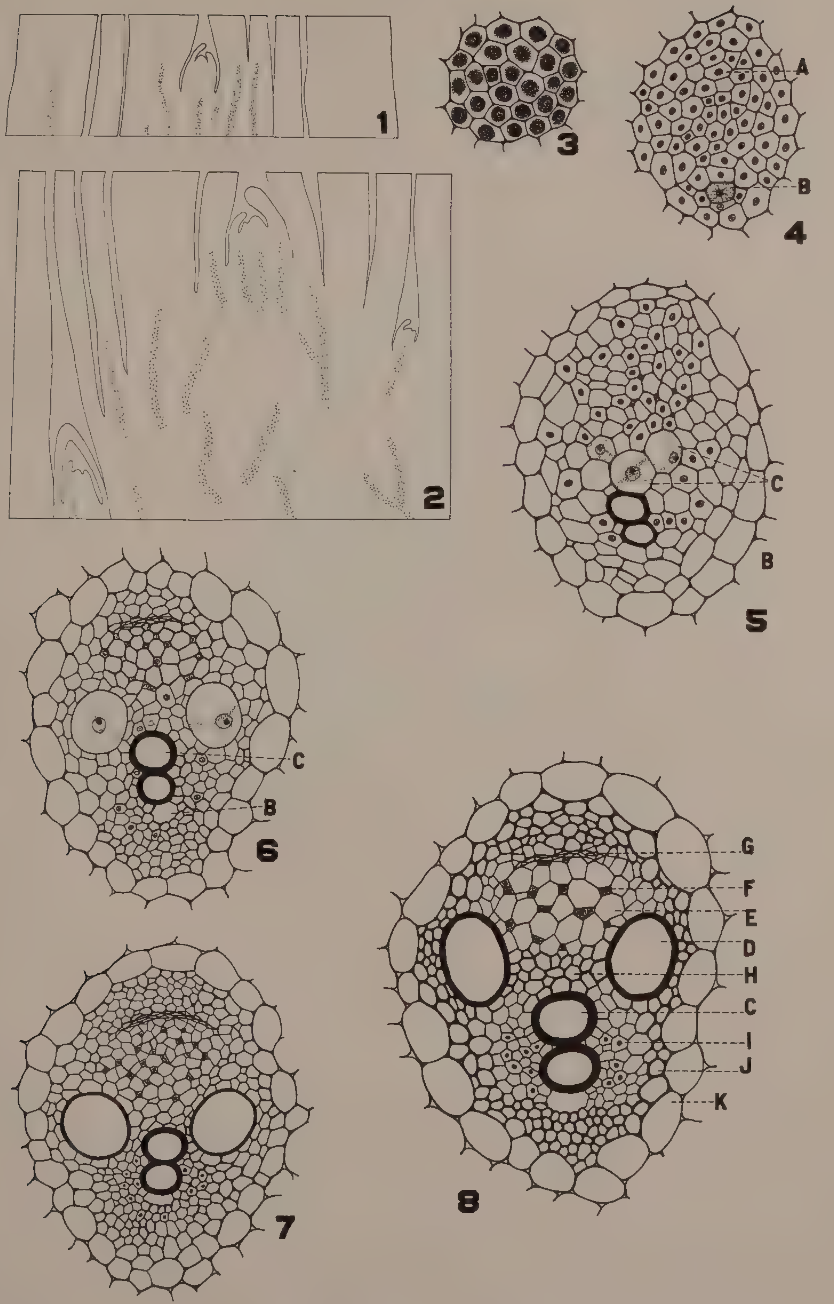

PLATE II

Floral primordia of terminal inflorescence, five weeks after planting. x 35.

PLATE II

NOTES ON NORTH AMERICAN SPIDERS OF THE FAMILIES GNAPHOSIDAE, ANYPHAENIDAE, AND CLUBIONIDAE

IRVING FOX

From the Department of Zoology and Entomology, Iowa State College

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The following report was made possible through the courtesy of the authorities of the United States National Museum, to whom I am indebted for the privilege of studying the collections of spiders in their charge. I wish also to express my appreciation to Dr. W. J. Gertsch of the American Museum of Natural History and Miss E. B. Bryant of the Museum of Comparative Zoology, who kindly lent material and gave valuable advice. Unless otherwise indicated the material on which this paper is based is in the United States National Museum.

In view of the scarcity of knowledge concerning the families under consideration, it is thought advisable to publish not only the descriptions of new species but also distributional notes which in most cases are new records for the states concerned.

Family Gnaphosidae

1. *Gnaphosa brumalis* Thorell

Gnaphosa brumalis Thorell, Proc. Boston Soc., N. H., 17: 497, 1877.

Records: Washington, Port Townsend, female; Labrador, two males and two females; Alaska, Fort Yukon, female.

2. *Gnaphosa fontinalis* Keyserling

Gnaphosa fontinalis Keyserling, Verh. Zoo. Bot. Ges. Wien, 37: 424, Pl. VI, fig. 4, 1887.

Record: Pennsylvania, York, female.

3. *Gnaphosa mima* Chamberlin

Gnaphosa mima Chamberlin, American Museum Novitates, No. 631, p. 2, figs. 3 and 4, 1933.

Record: Labrador, male.

4. *Gnaphosa parvula* Banks

Gnaphosa parvula Banks, Trans. Amer. Ent. Soc., 23: 61, 1893.

Records: Alberta, Calgary, October 30, 1927, female; Bilby, May 21, 1924, female (Owen Bryant, collector); Labrador, female; Nebraska, female.

5. *Gnaphosa gosoga* Chamberlin

Gnaphosa gosoga Chamberlin, Proc. Biol. Soc. Washington, 41: 178, 1928.

Record: California, San Diego, female.

6. *Gnaphosa sericata* L. Koch

Gnaphosa sericata L. Koch, Arachn. Fam. der Drassiden, p. 31, Pl. ii, fig. 21, 1866.

Records: New Brunswick, St. John, female; Labrador, two females; Utah, male and female; Washington, D. C., male.

7. *Gnaphosa hirsutipes* Bks.

Plate I, fig. 9

Gnaphosa hirsutipes Banks, Proc. Acad. Nat. Sci. Phil., 53: 573, Pl. XXXIII, fig. 4, 1901.

Male. Total length, 9.0 mm. Carapace, 3.6 mm. long, 2.9 mm. at the widest place, 1.8 mm. wide in front. Carapace orange yellow above, the pars cephalica and chelicerae darker than the pars thoracica. Sternum orange brown, the coxae lighter, labium and endites dark brown. Legs pale, lighter than the carapace, the tarsi somewhat darker than the other joints. Dorsum of the abdomen grayish with four dark punctuations on the basal half; venter concolorous with the dorsum.

Anterior row of eyes slightly procurved, narrower than the recurved posterior row (11/16). Anterior median eyes three-fourths as large as the anterior lateral, closer to the latter than to each other, separated from each other by two-thirds of a diameter. Posterior lateral eyes oblique, the long diameter longer than the diameter of the circular posterior lateral eyes (4/3), closer to each other than to the posterior lateral eyes being removed from each other by about one-half a long diameter, from the posterior lateral by more than a long diameter. Median ocular quadrangle longer than wide (9/10), somewhat narrower in front than behind. Clypeus higher than the diameter of an anterior median eye (5/3). Lower margin of the chelicerae armed with a distinct keel whose constituent teeth are not distinct. Anterior tibiae and metatarsi without spines below; posterior tibiae with 2-2-2 spines below and posterior metatarsi with a pair of basal spines and a ring of spines distally. Tibia and patella I, 4.6 mm. long (tibia alone, 2.8 mm.); tibia and patella II, 4.2 mm. (tibia alone, 2.7 mm.). For details of the palpal organ see Plate I, fig. 9.

Described from a male specimen taken in Stoneham, Pawnee Buttes, Colorado, by M. Koerner and in the American Museum of Natural History. In the United States National Museum is a female from Denver, Colorado, and a female from San Jacinto, California.

8. **Gnaphosa septentrionalis,** n. sp.

Plate I, figs. 2 and 8

Male. Total length, 3.9 mm. Carapace, 2.1 mm. long, 1.6 mm. wide, Carapace light reddish brown above with dark striations extending from the dorsal groove. Chelicerae concolorous with the carapace, the labium somewhat darker. Legs clear, light reddish, paler than the carapace, armed with numerous setae and spines, abdomen dark gray above, with a basal longitudinal band which is flanked on each side by a pair of dark punctations. Sides and venter of the abdomen concolorous with the dorsum.

Anterior and posterior rows of eyes recurved, the posterior row broader than the anterior (13/8). Anterior median eyes two-thirds as large as the anterior lateral, almost contiguous with the latter but separated from each other by about a diameter. Posterior median eyes oblique, much longer than wide, the long diameter longer than the diameter of the circular posterior lateral eyes (4/3). Posterior lateral eyes removed from the posterior median eyes by about one diameter. Median ocular quadrangle slightly wider than long, slightly narrower in front than behind. Clypeus equal in height to the diameter of an anterior median eye. Keel of the lower cheliceral margin uneven, evidently consisting of several fused teeth. Tibiae I armed below with a single apical pair of spines, tibiae II armed below with an apical pair and single submedian spine, tibiae III and IV armed below with three pairs of spines. Metatarsi I armed with a single basal spine, metatarsi II armed with a basal pair of spines. Tibia and patella I as long as tibia and patella IV. For the structure of the palpal organ see Plate I, fig. 2.

Female: Total length, 7.2 mm. Carapace, 2.64 mm. long, 1.98 mm. at the widest place, 1.2 mm. wide in front. Carapace and chelicerae dark reddish brown above with indications of dark striations extending from the dorsal groove. Sides concolorous with the dorsum lacking distinct submarginal stripes. Sternum, coxae, labium, and endites dark reddish brown, the legs somewhat lighter. Dorsum of the abdomen light brown above without any dark markings, sides and venter concolorous with the dorsum.

Anterior row of eyes straight or slightly recurved, much narrower than the recurved posterior row (12/16). Anterior median eyes slightly closer to the anterior lateral than to each other and about two-thirds as large as the latter. Posterior median eyes closer to each other than to the posterior lateral eyes, much more circular than in the male, separated from each other by about one-half a long diameter, from the posterior lateral by more than a long diameter. Median ocular quadrangle slightly longer than wide, narrower in front than behind. Clypeus about as high as the diameter of an anterior median eye. Lower cheliceral margin armed with a distinct keel consisting of five or six fused teeth. Tibiae I without spines below, tibiae II with a single apical pair, tibiae III and IV with three pairs of spines below. Tibia and patella I, 2.1 mm. long (tibia alone 1.2 mm.), tibia and patella IV, 2.5 mm. long (tibia alone, 1.5 mm.). For the structure of the epigynum see Plate I, fig. 8.

Type locality: Alaska: Male holotype from Schumaline Island and female allotype from Allognagik (no further data).

Type: U. S. N. M. Cat. No. 1286.

This new species resembles *G. hirsutipes* Banks, but may be distinguished from the latter by the structure of the copulatory organs.

9. **Gnaphosa tenebrosa,** n sp.

Plate II, fig. 6

Female. Total length, 6.8 mm. Carapace, 2.7 mm. long, 1.82 mm. at widest place, 1.2 mm. wide in front. Carapace dark reddish brown above, lighter at the median part, with indications of dark striations extending from the dorsal groove. Sides concolorous with the dorsum, without sub-

marginal stripes. Sternum, labium, and endites dark reddish brown, coxae much lighter. Legs light reddish brown, uniform, without annulations. Dorsum, sides and venter of the abdomen lighter than the carapace, the venter with two dark longitudinal lines.

Anterior and posterior rows of eyes recurved, the anterior row narrower than the posterior (9/11). Anterior median eyes closer to the anterior lateral than to each other and about two-thirds as large as the latter. Posterior median eyes closer to each oher than to the posterior lateral, oblique, separated from the posterior lateral by less than a long diameter, from each other by about one-half a long diameter. Median ocular quadrangle about as long as wide, about as wide in front as behind. Clypeus about as high as the diameter of an anterior median eye. Lower cheliceral margin armed with a distinct keel whose distal end is broken into several points. Tibiae I and II without spines below. Tibia and patella I, 2.16 mm. long (tibia alone, 1.26 mm.); tibia and patella IV, 2.16 mm. long (tibia alone, 1.26 mm.). For the structure of the epigynum see Plate II, fig. 6.

Type locality: Female holotype from Labrador (no further data).

Type: U. S. N. M. Cat. No. 1287.

10. **Gnaphosa subparvula**, n. sp.

Plate II, fig. 5

Female: Total length, 6.0 mm. Carapace, 3.3 mm. long, 2.4 mm. at the widest place, 2.2 mm. wide in front. Carapace dusky gray above, darker at the junction of the pars thoracica with the pars cephalica. Sides and margins of the carapace much darker than the dorsum. Sternum, labium, endites, and coxae light yellowish to dark brown. Legs concolorous with the dorsum, the femora darker. Dorsum and sides of the abdomen darker than the carapace; venter lighter.

Anterior row of eyes procurved, narrower than the recurved posterior row (8/11). Anterior median eyes closer to the anterior lateral than to each other, separated from each other by about a diameter, from the anterior lateral by about one-half a diameter, about two-thirds as large as the anterior lateral. Posterior median eyes almost circular, closer to each other than to the posterior lateral, separated from each other by two-thirds a long diameter, from the posterior lateral by slightly more than a long diameter. Posterior median eyes about two-thirds as large as the posterior lateral. Median ocular quadrangle slightly wider than long, slightly narrower in front than behind. Clypeus about twice as high as the diameter of an anterior median eye. Lower cheliceral margin armed with a distinct keel whose distal edge is characterized by a triangular indentation, and whose constituent teeth are indistinct. Tibiae I and II with a distal pair of spines and sometimes with a single submedian spine in addition. Metatarsi I and II armed with a submedian pair of spines. Tibia and patella I, 2.4 mm. long (tibia alone, 1.4 mm.); tibia and patella IV, 2.3 mm. long (tibia alone, 1.6 mm.). Epigynum as shown in Plate II, fig. 5.

Type locality: Female holotype and three female paratypes from Labrador (no further data).

Type: U. S. N. M. Cat. No. 1288.

This new species differs from *G. parvula* Banks in the lesser development of the process extending caudad from the anterior border of the epigynum and in the spination of the anterior tibiae.

11. **Gnaphosa labradorensis,** n. sp.

Plate I, fig. 1

Male: Total length, 6.0 mm. Carapace, 2.8 mm. long, 2.2 mm. at the widest place, 1.0 mm. wide in front. The specimen at hand is very old and in bad condition, hence the following description may not strictly apply to all individuals of the species. Carapace dark brown above without a relieving pattern. Sternum, labium, and coxae concolorous with the dorsum of the carapace, endites much lighter. Legs dark brown, somewhat lighter than the carapace. Abdomen indescribable because of dessication.

Anterior row of eyes slightly procurved, narrower than the recurved posterior row (8/11). Anterior median eyes closer to the anterior lateral than to each other and much smaller than the latter. Posterior median eyes almost circular, much closer to each other than to the posterior lateral eyes, and separated from the latter by more than a diameter. Median ocular quadrangle about as long as wide and about as wide in front as behind. Clypeus about twice as high as the diameter of an anterior median eye. Lower cheliceral margin armed with a distinct keel whose distal edge is broken into points and whose constituent teeth are very distinct. Tibiae I and II apparently with a distal pair of spines. Metatarsi I and II apparently with a single submedian pair of spines. Tibia and patella I equal in size to tibia and patella II. Tibia and patella I, 2.7 mm. (tibia alone, 1.5 mm.). For the structure of the male palpus see Plate I, fig. 1.

Type locality: Male holotype from Labrador (no further data).

Type: U. S. N. M. Cat. No. 1289.

The species described above bears a close resemblance to *G. parvula* Banks, especially in the structure of the palpal organ, but may be distinguished from the latter particularly by the greater development of the tibial apophysis.

12. *Herpyllus hesperolus* Chamberlin

Herpyllus hesperolus Chamberlin, Proc. Bio. Soc. Washington, 41:176, 1928.

Record: Utah, male and female.

13. *Herpyllus propinquus* (Keyserling)

Prosthesima propinqua Keyserling, Verh. Zool. Bot. Ges. Wien, 37:430. Pl. VI, fig. 7, 1887.

Herpyllus propinquus Chamberlin, Proc. Bio. Soc. Washington, 35:149, 1922.

Record: Lake Superior, female; California, San Diego, male and female.

14. *Herpyllus vasifer* (Walckenaer)

Drassylus vasifer Walckenaer, Tabl. Aran. p. 46, 1805.

Herpyllus vasifer Petrunkevitch, Bull. American Mus. Nat. Hist., 29:144, 1911.

Records: Utah, 2 males; Washington, D. C., 2 males; Nebraska, male; Indiana, male and female; Wyoming, Fort Bridges, female; California, San Francisco, female; Georgia, Atlanta, female; Colorado, Pikes Peak, female; Iowa, Ames, male (E. R. Becker, collector); Florida, male.

15. **Herpyllus bensonae, n. sp.**

Plate I, fig. 7

Male: Total length, 8.2 mm. Carapace, 4.2 mm. long, 3.0 mm. at the widest place, 1.6 mm. wide in front. Carapace reddish brown above, the pars cephalica somewhat lighter than the pars thoracica. Labium, endites, and coxae also reddish brown, sternum somewhat lighter. Legs dark brown, concolorous with the dorsum of the carapace. Abdomen light reddish brown, basal half with several irregular punctations arranged in two rows. The abdomen is cornified basally. Venter concolorous with the dorsum, with two longitudinal dark lines extending from the epigastric furrow almost to the spinnerets.

Anterior row of eyes slightly recurved, somewhat narrower than the slightly procurved posterior row (15/16). Anterior median eyes separated from each other by a diameter, contiguous with the anterior lateral eyes and much larger than the latter (7/5). Eyes of the posterior row subequidistant, the posterior median eyes about five-sixths as large as the posterior lateral. Median ocular quadrangle slightly wider than long, wider in front than behind. Clypeus equal in height to about one and one-half times the diameter of an anterior median eye. Lower cheliceral margin unarmed below, upper margin apparently armed with two fused teeth. Tibiae I armed with 2-2-2 spines below, metatarsi I armed with a single basal pair of spines below. Tibia and patella I, 4.5 mm. long (tibia alone, 2.7 mm.); tibia and patella IV missing. For the structure of the palpal organ see Plate I, fig. 7.

Type locality: Male holotype from Washington, D. C. (no further data).

Type: U. S. N. M. Cat. No. 1290.

The palpal organ of this new species bears considerable resemblance to that of *G. hesperolus* Chamberlin, but may be distinguished from it by the structure of the tibial apophysis which is not bibranchiate.

16. **Herpyllus excelsus, n. sp.**

Plate II, fig. 8

Female: Total length, 10.4 mm. Carapace, 3.3 mm. long, 2.4 mm. at the widest place, 1.3 mm. wide in front. Carapace light reddish brown without distinct markings. Sternum, legs, and endites concolorous with the dorsum of the carapace, labium somewhat darker. Dorsum of the abdomen, sides, and venter pale gray, without dark markings.

Anterior row of eyes slightly recurved, narrower than the procurved posterior row (12/14). Anterior median eyes separated from each other by about two-thirds of a diameter, almost contiguous with the anterior lateral eyes and much larger than the latter (3/2). Eyes of the posterior row subequidistant, the posterior median eyes about two-thirds as large as the posterior lateral. Median ocular quadrangle slightly longer than wide, wider in front than behind (8/6). Clypeus equal in height to about one-half the diameter of an anterior median eye. Lower cheliceral margin with indications of a single poorly developed tooth, upper margin with two well-developed teeth and indications of a third. Tibiae I armed with 1-1-1 spines, tibiae II apparently armed with 2-2 spines. Metatarsi I armed

with a single basal spine, metatarsi II armed with a basal pair of spines. Tibia and patella I, 3.2 mm. long (tibia alone, 1.92 mm.); tibia and patella IV, 3.7 mm. long (tibia alone, 2.4 mm.). For the structure of the epigynum see Plate II, fig. 8.

Type locality: Arizona: Female holotype from Chiricahua Mts. (no further data).

Type: U. S. N. M. Cat. No. 1291.

17. **Herpyllus australis, n. sp.**

Plate II, fig. 1

Female: Total length, 8.4 mm. Carapace, 2.8 mm. long, 2.0 mm. at the widest place, 1.2 mm. wide in front. Dorsum of the carapace dark reddish brown above, sides with dark irregular submarginal bands. Sternum and coxae light reddish brown, lighter than the carapace, labium and endites concolorous with the carapace. Legs light reddish brown, femora somewhat darker than the other joints. Dorsum and sides of the abdomen gray, venter with indications of two dark longitudinal lines extending from the epigastric furrow almost to the spinnerets.

Anterior and posterior rows of eyes slightly recurved, the anterior row narrower than the posterior row (19/22). Anterior median eyes closer to the anterior lateral than to each other, separated from each other by about three-fifths of a diameter, almost contiguous with the anterior lateral and larger than the latter (5/4). Posterior median eye closer to the posterior lateral than to each other, separated from each other by more than two diameters, removed from the posterior lateral by more than a diameter and about one-half as large as the latter. Median ocular quadrangle somewhat longer than wide, about as wide in front as behind. Lateral eyes of each row unusually wide apart, being separated by more than the diameter of a posterior lateral eye. Clypeus equal in height to the diameter of an anterior median eye. Lower cheliceral margin with indications of a weak tooth, upper margin armed with two robust teeth and indications of a third. Tibiae I armed with 1-1-1 spines below, tibiae II armed with 0-1-1 spines below. Metatarsi I and II armed with a basal pair of spines. Tibia and patella I, 2.4 mm. long (tibia alone, 1.4 mm.); tibia and patella IV, 2.7 mm. long (tibia alone, 1.5 mm.). For the structure of the epigynum see Plate II, fig. 1.

Type locality: Florida: female holotype from Key West (no further data).

Type: U. S. N. M. Cat. No. 1292.

In the arrangement of the eyes the new species described above differs somewhat from most species of *Herpyllus.* Its general structure and epigynum, however, are characteristic of that genus.

18. *Haplodrassus taibo* (Chamberlin)

Zelotes taibo Chamberlin, Pomona Jour. Ent. and Zool., 12:6, Pl. 2, fig. 5, 1920 (adv. reprint, 1919).

Haplodrassus taibo Chamberlin, Proc. Bio. Soc. Washington, 35:161, 1922.

Record: Utah, female.

19. *Haplodrassus magister* Chamberlin

Haplodrassus magister Chamberlin, Amer. Mus. Novit. No. 631, Pl. 6, figs. 11 and 12, 1933.

Record: Labrador, male.

20. *Drassylus arizonensis* (Banks), new comb.

Plate II, fig. 3

Prosthesima arizonensis Banks, Proc. United States Nat. Mus., 23: 582, 1901.

Zelotes (?) *arizonensis* Chamberlin, Proc. Bio. Soc. Washington, 35: 166, 1922.

The holotype of the above species is in the United States National Museum.

Type: U. S. N. M., Cat. No. 5424.

21. *Drassylus aprilinus* (Banks)

Zelotes aprilinus Banks, Jour. New York Ent. Soc., 12: 110, Pl. 5, fig. 7, 1904.

Drassylus aprilinus Chamberlin, Proc. Bio. Soc. Washington, 35: 170, 1922.

Record: Washington, D. C., female.

22. *Drassylus depressus* (Emerton)

Prosthesima depressa Emerton, Trans. Conn. Acad., 8: 173, Pl. III, fig. 8, 1889.

Drassylus depressus Chamberlin, Proc. Bio. Soc. Washington, 35: 167, 1922.

Records: New Mexico, female; Pennsylvania, male.

23. *Drassylus frigidus* (Banks)

Prosthesima frigida Banks, Proc. Acad. Philadelphia, p. 17, Pl. I, fig. 56, 1892.

Drassylus frigidus Chamberlin, Proc. Bio. Soc. Washington, 35: 168, 1922.

Records: Washington, D. C., male and female; Maryland, Baltimore, male and female.

24. *Drassylus rationalis* Chamberlin

Drassylus rationalis Chamberlin, Proc. California Acad., 12: 629, fig. 67, 1924.

Records: California, San Diego, female; San Francisco, female.

25. *Drassylus rufulus* (Banks)

Prosthesima rufula Banks, Proc. Acad. Philadelphia, p. 17, Pl. I, fig. 55, 1892.

Drassylus rufulus Chamberlin, Proc. Bio. Soc. Washington, 35: 167, 1922.

Records: Nebraska, Lincoln, female; Colorado, Pikes Peak, two females; Pennsylvania, Philadelphia, female.

26. *Drassylus saphes* Chamberlin

Drassylus saphes Chamberlin, Amer. Mus. Novit., No. 841, p. 29, fig. 44, 1936.
Record: Arizona, Phoenix, female.

27. *Poecilochroa montana* Emerton

Poecilochroa montana Emerton, Trans. Connecticut Acad. Sci., 8: 175, Pl. IV, fig. 2, 1889.
Record: Washington, D. C., female.

28. *Poecilochroa columbiana* Emerton

Poecilochroa columbiana Emerton, Can. Ent., 49: 269, fig. 21, 1917.
Record: California, San Diego, female.

29. *Drassodes neglectus* (Keyserling)

Drassus neglectus Keyserling, Verh. Zool. Bot. Ges. Wien, 37: 434, fig. 10, 1887.
Drassodes neglectus Petrunkevitch, Bull. Amer. Mus. Nat. Hist., 29: 138, 1911.
Record: Florida, female; Oregon, Lake Klamath, male; Alberta, Edmonton, May 10, 1924 (Owen Bryant, collector), male.

30. *Drassodes robinsoni* Chamberlin

Drassodes robinsoni Chamberlin, Ann. Ent. Soc. America, 12: 245, Pl. 16, fig. 2, 1919.
Record: North Carolina, Chapel Hill, March 27, 1886, female; British Columbia, Kamloops, Oct. 26, 1929 (O. Bryant, collector), female.

31. *Drassodes celes* Chamberlin

Drassodes celes Chamberlin, Pomona Jour. Ent. and Zool., 12: 5, Pl. 2, fig. 2, 1920 (adv. reprint, 1919).
Records: California, San Jacinto, female; Texas, Columbus, female.

32. *Litopyllus luteus* (Barrows)

Prosthesima lutea Barrows, Ohio Jour. Sci., 19: 356, Pl. XV, fig. 5, 1919.
Litopyllus luteus Chamberlin, Proc. Bio. Soc. Washington, 35: 155, 1922.
Record: Washington, D. C., male and female.

33. **Litopyllus ambiguus,** n. sp.

Plate I, fig. 4

Male: Total length, 5.8 mm. Carapace, 2.8 mm. long, 2.1 mm. at the widest place, 1.0 mm. wide in front. Carapace reddish brown without distinct lighter markings. Sternum, coxae, endites, light reddish, labium

somewhat darker. Legs light reddish brown, concolorous with the sternum. Abdomen reddish brown, the basal portion cornified, with two rows of punctations consisting of three each at about the middle third. Venter lighter than the dorsum and without dark lines.

Anterior row of eyes slightly recurved, about as long as the procurved posterior row. Anterior median eyes larger than the anterior lateral, almost contiguous with the latter but separated from each other by about one-half a diameter. Posterior median eyes larger than the posterior lateral, oblique, much closer to each other than to the posterior lateral. Median ocular quadrangle slightly longer than wide, wider in front than behind. Clypeus equal in height to the diameter of an anterior median eye. Tibiae I armed with 1-1-1 spines below; metatarsi I armed with a basal pair of spines. Tibia and patella I, 2.8 mm. long (tibia alone, 1.6 mm.); tibia and patella II, 3.0 mm. long (tibia alone, 1.9 mm.). For the structure of the palpal organ see Plate I, fig. 4.

Type locality: Male holotype from New Mexico (no further data).

Type: U. S. N. M. Cat. No. 1293.

34. **Sosticus projectus**, n. sp.

Plate I, figures 3 and 5

Female: Total length, 9.8 mm. Carapace, 3.3 mm. long, 2.6 mm. at the widest place, 1.4 mm. wide in front. Carapace dark reddish brown without lighter markings. Sternum dark reddish brown at the edges, lighter in the center; coxae and endites light reddish brown, lighter than the sternum and the carapace. Legs reddish brown with the femora somewhat darker than the other joints. Dorsum of the carapace gray, basally with two rows of punctations consisting of three each; venter lighter than the dorsum with two dark longitudinal lines extending from the epigastric furrow almost to the spinnerets.

Anterior row of eyes procurved, narrower than the straight posterior row (11/13). Anterior median eyes about as large as the anterior lateral, closer to the latter than to each other, removed from each other by less than one-half a diameter. Posterior median eyes smaller than the posterior lateral, closer to the latter than to each other, removed from each other by about a diameter. Median ocular quadrangle as wide as long, as wide in front as behind. Clypeus slightly higher than the diameter of an anterior median eye. Lower cheliceral margin armed with two teeth, upper margin armed with three. Tibiae I unarmed below; tibiae II with a single submedian spine. Metatarsi I armed with a basal pair of spines, metatarsi II armed with a basal pair and a submedian spine. Tibia and patella I, 3.2 mm. long (tibia alone, 1.8 mm.); tibia and patella IV, 3.6 mm. long (tibia alone, 2.2 mm.).

Epigynum much longer than wide (10/15), provided with a posteriorly projecting heavily chitinized scape. For further details of the structure of the epigynum see Plate I, fig. 5.

Male: Total length, 5.8 mm. Carapace, 2.8 mm. long, 2.3 mm. at the widest place, 1.1 mm. wide in front. Carapace dark reddish brown above with a distinct light patch at the posterior portion of the pars cephalica. Sternum, coxae, and endites light orange brown, the labium somewhat darker. Legs reddish brown, the femora darker than the other joints.

Abdomen irregular dark reddish brown above, the basal portion much lighter; venter concolorous with the sternum, with two dark longitudinal lines extending from the epigastric furrow to the spinnerets.

Anterior row of eyes recurved, narrower than the procurved posterior row (9/11). Other eye arrangements essentially as in the female. Median ocular quadrangle slightly larger than wide, about as wide in front as behind. Lower cheliceral margin armed with two teeth, upper with three. Clypeus equal in height to about twice the diameter of an anterior median eye. Tibiae I and II armed with 2-2-2 spines below, metatarsi I and II armed with 2-2 spines. Tibia and patella I, 2.5 mm. long (tibia alone, 1.7 mm.); tibia and patella IV, 3.1 mm. long (tibia alone, 1.9 mm.). For the structure of the palpae organ see Plate I, fig. 3.

Type locality: Female holotype and male allotype from Indiana (no further data).

Type: U. S. N. M. Cat. No. 1294.

35. **Zelotes pullatus**, n. sp.

Plate II, fig. 2

Female: Total length, 9.6 mm. Carapace, 3.6 mm. long, 2.9 mm. at the widest place, 1.6 mm. wide in front. Carapace, sternum, endites, labium, and legs dark brown to black. Dorsum of the abdomen and sides gray without distinct markings. Venter somewhat lighter.

Anterior row of eyes slightly recurved, narrower than the straight posterior row (9/11). Anterior median eyes closer to the anterior lateral than to each other and about two-thirds as large as the latter. Eyes of the posterior row subequidistant, the posterior median eyes about two-thirds as large as the posterior lateral. Median ocular quadrangle slightly wider than long, as wide in front as behind. Clypeus equal in height to almost twice the diameter of an anterior median eye. Lower cheliceral margin armed with one tooth, upper margin armed with three teeth. Tibiae I and II unarmed below; metatarsi I and II with a basal pair of spines. Tibia and patella I, 3.7 mm. long (tibia alone, 2.1 mm.); tibia and patella IV, 3.9 mm. long (tibia alone, 2.4 mm.). Epigynum longer than wide (22/15) resembling that of *Z. subterreaneus* (C. Koch) from which it may be distinguished by the details shown in Plate II, fig. 2.

Type locality: Female holotype from Kamloops, British Columbia, October 26, 1929, (O. Bryant, collector).

Type: U. S. N. M. Cat. No. 1295.

36. *Orodrassus coloradensis* (Emerton)

Drassus coloradensis Emerton, Bull. United States Geol. Survey, 3: 528, Fig. 19, 1877.

Orodrassus coloradensis Chamberlin, Proc. Bio. Soc. Washington, 35: 163, 1922.

Records: Colorado, West Cliff, two females (T. D. A. Cockerell, collector); Alberta, Banff, May 9, 1928, female; Calgary, female (O. Bryant, collector).

37. *Orodrassus durranti* Chamberlin

Orodrassus durranti Chamberlin, American Mus. Novit. No. 853, p. 7, Fig. 22, 1936.

Record: Oregon, Lake Klamath, female.

38. *Orodrassus vastus* (Banks)

Drassus vastus Banks, Trans. American Ent. Soc., 23: 63, 1896.

Orodrassus vastus Chamberlin, Proc. Bio. Soc. Washington, 35: 163, 1922.

Records: Alberta, Edmonton, April 2, 1924, female; Bilby, August, 1924, female (O. Bryant, collector).

Family Anyphaenidae

39. *Aysha decepta* (Banks)

Anyphaena decepta Banks, Proc. Ent. Soc. Washington, 4: 190, 1896.

Aysha decepta Bryant, Psyche, 38: 121, Figs. 16, 27, 1931.

Record: Colorado, Pikes Peak, female.

40. *Anyphaena aperta* (Banks)

Plate I, fig. 6

Gayenna aperta Banks, Proc. California Acad. Sci. 11: 100, Fig.3, 1921.

Anyphaena aperta Bryant, Psyche, 38: 114, Fig. 35, 1931.

Male: Total length, 4.5 mm. Carapace, 1.8 mm. long, 1.5 mm. at the widest place, .8 mm. wide in front. Carapace dark reddish brown with irregular light spots and streaks, the pars cephalica much lighter than the rest of the carapace. Sternum and endites light reddish brown, coxae and labium lighter. Legs reddish brown with irregular annulations at the distal ends of the femora and the proximal ends of the tibiae and metatarsi. Abdomen yellowish with dark brown streaks and spots; venter without distinct markings. Spiracle situated in the middle region of the abdomen, but slightly closer to the spinnerets than to the epigastric furrow.

Anterior row of eyes slightly recurved, narrower than the procurved posterior row (11/14). Anterior median eyes about two-thirds as large as the anterior lateral and closer to the latter than to each other. Eyes of the posterior row subequal, the anterior median eyes removed from each other by about a diameter, much closer to the anterior lateral. Median ocular quadrangle about as long as wide, narrower in front than behind (5/7). Clypeus equal in height to about one-half the diameter of an anterior median eye. Tibiae I and II armed with 2-2-2 spines below; metatarsi I and II armed with a basal pair of spines. Tibia and patella I about equal in length to tibia and patella IV. Tibia and patella I, 2.4 mm. long (tibia alone, 1.4 mm.). For details regarding the structure of the palpal organ see Plate I, fig. 6.

Records: California, San Francisco, two males and a female; Washington, Tinino, male and female; Oregon, Astoria, male.

Family Clubionidae

41. *Agroeca pratensis* Emerton

Agroeca pratensis Emerton, Trans. Connecticut Acad. Sci., 8: 190, P1. VI, fig. 7, 1889.

Records: Washington, D. C., 2 females; British Columbia, Kamloops, October 26, 1929 (O. Bryant, collector), female.

42. *Clubiona abboti* L. Koch

Clubiona abboti L. Koch, Die Arach. Fam. der Drassiden, p. 303, Pl. XII, fig. 193, 1866.
Record: Alberta, Beaver Lake, May 25, 1924, female (O. Bryant, collector).

43. *Clubiona canadensis* Emerton

Clubiona canadensis Emerton, Trans. Connecticut Acad. Sci., 8:181, Pl. V, fig. 4, 1889.
Records: Alberta, Banff, July, 1925, female (O. Bryant, collector): Labrador, female; Aleutian Islands, female.

44. *Clubiona minutissima* Petrunkevitch

Clubiona minutissima Petrunkevitch, Bull. American Mus. Nat. Hist., 29:461, 1911.
Record: Washington, D. C., two males.

45. *Clubiona tibialis* Emerton

Clubiona tibilis Emerton, Trans. Connecticut Acad. Sci., 8:180, P1. V, fig. 3, 1889.
Records: Minnesota, St. Paul, female; Washington, D. C., males and females; Alabama, Selma, female.

46. **Clubiona californica,** n. sp.

Plate II, fig. 4.

Female: Total length, 7.5 mm. Carapace, 2.8 mm. long, 2.1 mm. at the widest place, 1.6 mm. wide in front. Carapace dark reddish brown, pars cephalica with an indistinct median light longitudinal band. Sternum and coxae light reddish brown, labium and endites much darker. Legs reddish brown, lighter than the carapace, the femora darker than the other joints. Abdomen light reddish brown, the dorsum with dark median lanceolate band extending from the anterior end to a point about half way down the length of the abdomen; venter unmarked.

Anterior row of eyes straight, narrower than the procurved posterior row (10/15). Eyes of the anterior row subequal and equidistant. Posterior median eyes somewhat closer to the posterior lateral than to each other and about equal to them in size. Median ocular quadrangle about as long as wide, much narrower in front than behind (7/10). Clypeus equal in height to one-half the diameter of an anterior median eye. Lower cheliceral margin armed with two robust teeth, upper margin armed with five teeth of which one is strong while the other four are weak. Tibiae I and II armed with 2-2 spines below. Metatarsi I and II armed with a basal pair of spines below, tibia and patella I, 2.4 mm. long (tibia alone, 1.5 mm.); tibia and patella IV, 3.0 mm. long (tibia alone, 1.9 mm.). For the structure of the epigynum see Plate II, fig. 4.

Type locality: Female holotype from San Francisco, California.
Type: U. S. N. M. Cat. No. 1296.

47. **Clubiona carpenterae**, n. sp.

Plate II, fig. 7.

Female: Total length, 5.7 mm. Carapace, 2.5 mm. long, 1.8 mm. at the widest place, 1.3 mm. wide in front. Carapace dark reddish brown above, the eye region much lighter, pars cephalica with an indistinct median light band. Sternum and coxae light yellowish brown, labium and endites much darker. Legs light orange brown, contrasting with the dark carapace. Abdomen reddish brown above with a basal median lanceolate mark; venter light reddish brown without distinct markings.

Anterior row of eyes slightly recurved, narrower than the procurved posterior row (13/18). Anterior median eyes slightly smaller than the anterior lateral, about as far from the anterior lateral eyes as from each other. Eyes of the posterior row subequal, the posterior median closer to the posterior lateral than to each other. Median ocular quadrangle wider than long (12/7), much narrower in front than behind (7/12). Clypeus equal in height to one-half the diameter of an anterior median eye. Tibiae I and II armed with 2-2 spines below, metatarsi I and II armed with a basal pair of spines below. Tibia and patella I, 2.5 mm. long (tibia alone, 1.2 mm.); tibia and patella II, 2.7 mm. long (tibia alone, 1.7 mm.). Epigynum as shown in Plate II, fig. 7.

Type locality: Female holotype from Labrador (no further data).
Type: U. S. N. M. Cat. No. 1297.

Explanation of Plate I

Fig. 1—*Gnaphosa labradorensis,* n. sp., male, palpus
Fig. 2—*G. septentrionalis,* n. sp., male, palpus
Fig. 3—*Sosticus projectus,* n. sp., male, palpus
Fig. 4—*Litopyllus ambiguus,* n. sp., male, palpus
Fig. 5—*Sosticus projectus,* n. sp., epigynum
Fig. 6—*Anyphaena aperta* (Banks), male, palpus
Fig. 7—*Herpyllus bensonae,* n. sp., male, palpus
Fig. 8—*Gnaphosa septentrionalis,* n. sp., epigynum
Fig. 9—*G. hirsutipes* Banks, male, palpus

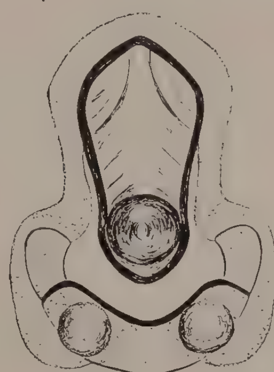
PLATE I


1


2


3


4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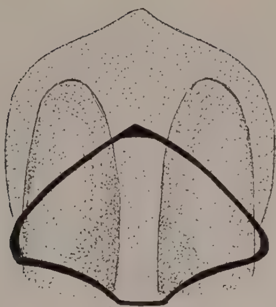
Explanation of Plate II

FIG. 1—*Herpyllus australis,* n. sp., epigynum
FIG. 2—*Zellotes pullatus,* n. sp., epigynum
FIG. 3—*Drassylus arizonensis* (Banks), epigynum
FIG. 4—*Clubiona californica,* n. sp., epigynum
FIG. 5—*Gnaphosa subparvula,* n. sp., epigynum
FIG 6—*Gnaphosa tenebrosa,* n. sp., epigynum
FIG. 7—*Clubiona carpenterae,* n. sp., epigynum
FIG. 8—*Herpyllus excelsus,* n. sp., epigynum

PLATE II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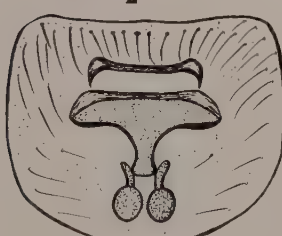
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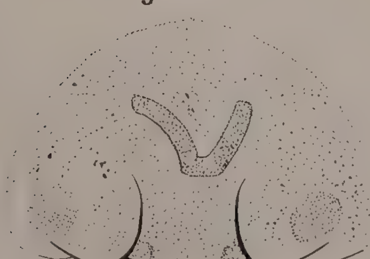
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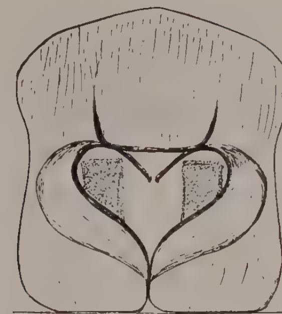
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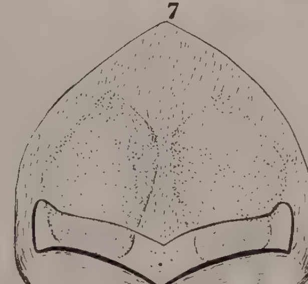
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BEHAVIOR OF BUTYRIC ACID-BUTYL ALCOHOL BACTERIA TOWARD ACETYLMETHYLCARBINOL AND ASPARAGIN[1]

R. W. Brown, G. L. Stahly and C. H. Werkman

From the Bacteriology Section, Iowa Agricultural Experiment Station

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Acetylmethylcarbinol is a common product of bacterial dissimilation; however, it is usually reduced to 2,3-butylene glycol and only traces of the carbinol occur in the fermentation. Even among the strongly aerobic organisms of the genus Bacillus, the reduction takes place and only traces of acetylmethylcarbinol can be found.

Harden and Walpole (1906) isolated and described both 2,3-butylene glycol and acetylmethylcarbinol from fermentations of glucose and mannitol by *Aerobacter aerogenes.* Harden and Norris (1912) found that *Aerobacter aerogenes* and *Aerobacter cloacae* when grown in a peptone solution containing any one of a series of sugars or polyalcohols, produce both acetylmethylcarbinol and 2,3-butylene glycol.

Relatively few investigators have reported on the formation of acetylmethylcarbinol and 2,3-butylene glycol by anaerobic bacteria. McCoy, Fred, Peterson and Hastings (1926) found by qualitative tests that the carbinol was formed by *Clostridium acetobutylicum* from a variety of carbohydrates.

Donker (1926) found that the Weizmann culture *(Cl. acetonigenum)* forms acetylmethylcarbinol equivalent to as much as 2.6 per cent of glucose fermented but he detected no 2,3-butylene glycol. *Cl. pectinovorum, Cl. Beijerinckii, Cl. Pasteurianum* and *Cl. saccharobutyricum* did not produce acetylmethylcarbinol, although small amounts of 2,3-butylene glycol were isolated.

Wilson, Peterson and Fred (1927) reported that acetylmethylcarbinol was formed by *Cl. acetobutylicum* as a regular end product, averaging from 300 to 400 mg. per liter.

The present study deals with the behavior of the butyric acid-butyl alcohol bacteria (1) with respect to their formation or utilization of acetylmethylcarbinol and 2,3-butylene glycol and (2) the influence of asparagin on the yield of butyl alcohol.

EXPERIMENTAL

A commercial sample of acetylmethylcarbinol was purified by mixing with cold anhydrous ether, thoroughly stirring and filtering under vacuum. A snow-white, odorless, crystalline product was obtained.

Quantiative determinations of acetylmethylcarbinol and 2,3-butylene glycol were made according to Stahly and Werkman (1936) and Brockmann and Werkman (1933), respectively.

The medium employed consisted of peptone one per cent, glucose one per cent and dipotassium phosphate two per cent.

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[1] Journal paper No. J-511 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project No. 572.

Two flasks of medium were inoculated with each strain. One of each pair of flasks received a definite quantity of acetylmethylcarbinol which had been sterilized in water solution in an air-tight flask to prevent volatilization. The flasks were incubated in McIntosh and Fildes anaerobic jars at 37° C. At the conclusion of the fermentation the contents of the flasks were analyzed for acetylmethylcarbinol and 2,3-butylene glycol.

Qualitative tests for 2,3-butylene glycol were made by bromine oxidation and conversion to nickel dimethylglyoxime on cultures in which the acetylmethylcarbinol had disappeared. A precipitate of the glyoxime indicated the presence of the glycol.

The source of each culture used in this investigation was as follows: *Cl. acetobutylicum* B (1 C) and *Cl. acetobutylicum* K (3 C), Dr. Leo. F. Rettger, Yale University; *Cl. acetobutylicum* K (14 C), Dr. A. M. Wynne, University of Toronto; *Cl. acetobutylicum* K (F 1), (12 B) and (M S), Dr. L. M. Christensen, of the Chemical Foundation; *Cl. felsineum* (5 D), *Cl. butylicum* (2 D), *Cl. saccharobutyricum* (3 D), *Cl. Beijerinckii* (4 D),

TABLE 1. *Behavior of butyric acid–butyl alcohol bacteria toward acetylmethylcarbinol*

Culture number	Name	Acteylmethyl-carbinol added mM	Final products: Acetylmethyl-carbinol mM	Final products: 2, 3-Butylene glycol mM
2D	*Cl. butylicum*	0	0	00.9
2D	*Cl. butylicum*	14.8	0	15.1
2D	*Cl. butylicum*	0	0	00.7
2D	*Cl. butylicum*	14.8	0	14.6
6D	*Cl. Pasteurianum*	0	0	1.0
6D	*Cl. Pasteurianum*	14.8	0	15.3
6D	*Cl. Pasteurianum*	0	0	00.2
6D	*Cl. Pasteurianum*	14.8	0	14.4
10C	*Cl. pectinovorum*	0	0	1.0
10C	*Cl. pectinovorum*	20.2	0	19.1
4D	*Cl. Beijerinckii*	0	0	1.3
4D	*Cl. Beijerinckii*	16.0	0	15.2
3D	*Cl. saccharobutyricum*	0	0	1.3
3D	*Cl. saccharobutyricum*	20.2	0	19.1
5D	*Cl. felsineum*	0	1.1	00.3
5D	*Cl. felsineum*	20.2	21.4	0.0
MS	*Cl. acetobutylicum*	0	0.8	0.9
MS	*Cl. acetobutylicum*	40.7	46.6	......
Fl	*Cl. acetobutylicum*	0	1.3	0.0
Fl	*Cl. acetobutylicum*	20.2	21.7	0.0
14C	*Cl. acetobutylicum*	0	1.8	0.1
14C	*Cl. acetobutylicum*	20.2	21.6	00.0
1CB	*Cl. acetobutylicum*	0	1.0	0.0
1CB	*Cl. acetobutylicum*	14.8	13.5	0.0
1CB	*Cl. acetobutylicum*	0	1.3	00.0
1CB	*Cl. acetobutylicum*	16.0	16.4	0.0
3CK	*Cl. acetobutylicum*	0	1.1	......
3CK	*Cl. acetobutylicum*	40.7	46.1	0.9
12B	*Cl. acetobutylicum*	0	1.3	1.2
12B	*Cl. acetobutylicum*	40.7	46.7	0.4

TABLE 2. *Effect of asparagin on yields of "solvents" in corn mash*

Species	Culture number	Percentage total "solvents"	
		5 per cent corn mash	5 per cent corn mash plus 0.1 per cent asparagin
Cl. butylicum	1	0.00	23.12
Cl. butylicum	3	0.00	21.25
Cl. butylicum	6	0.00	20.44
Cl. butylicum	25	0.00	20.70
Cl. butylicum	43	0.00	24.73
Cl. butylicum	53	0.00	26.43
Cl. butylicum	2D	0.00	24.21
Cl. saccharobutyricum	3D	0.00	0.00
Cl. Beijerinckii	4D	0.00	0.00
Cl. Pasteurianum	6D	0.00	0.53
Cl. Pasteurianum	12C	0.00	0.26
Cl. pectinovorum	10C	0.00	00.00
Cl. acetobutylicum	1C	14.53	15.62
Cl. acetobutylicum	3C	20.44	19.36
Cl. acetobutylicum	14C	20.44	21.52
Cl. acetobutylicum	F1	22.05	21.52
Cl. acetobutylicum	MS	20.98	19.80
Cl. acetobutylicum	12B	22.59	21.52
Cl. felsineum	5D	22.59	20.98

Cl. Pasteurianum (6 D), Prof. A. J. Kluyver, Delft, Holland; *Cl. pectinovorum* 859 (10 C), American Type Culture Collection; *Cl. butylicum* (1), (3), (6), and (25), isolated from silage; *Cl. butylicum* (43) and (53), isolated from soil.

The results in table 1 show an interesting behavior of these anaerobic forms. A sharp division of the group occurs on the reduction of acetylmethylcarbinol to 2,3-butylene glycol. *Cl. acetobutylicum* and *Cl. felsineum*, forming one group, produce acetylmethylcarbinol but fail to reduce it to the glycol. This behavior is a little surprising in view of the known marked reducing ability of these organisms. Their reduction of butyric and acetic acids to the corresponding alcohols and of the latter acid to acetone is well known. Ability to reduce acetylmethylcarbinol is a widespread property of bacteria and its reduction is expected before that of butyric acid to butyl alcohol. The results were clear cut and there were no exceptions.

In the second subgroup were *Cl. butylicum, Cl. Pasteurianum, Cl. pectinovorum, Cl. accharobutyricum,* and *Cl. Beijerinckii.* All form 2,3-butylene glycol; added acetylmethylcarbinol is rapidly reduced to the glycol and the carbinol does not occur as an end product. It is apparent that in the dissimilation of glucose, acetylmethylcarbinol is formed as an intermediate product by these species. It is not present at the conclusion of the fermentation because it is reduced to the glycol as rapidly as formed. The presence of 2,3-butylene glycol is thus accounted for.

The behavior of the butyric acid-butyl alcohol bacteria with acetylmethylcarbinol provides a convenient and rapid method for their differentiation into two groups by testing for the presence of acetylmethylcarbinol

in fermented glucose media. If the test is positive the organism may be grouped with *Cl. acetobutylicum* and *Cl. felsineum;* the orange color of the latter differentiates it from *Cl. acetobutylicum.* If the test is negative, the organism belongs to the larger group comprising typical butyric acid bacteria. Confirmation may be made by adding suitable quantities of acetylmethylcarbinol to the medium and testing for its reduction after a few hours. However, it is not implied that the test for acetylmethylcarbinol differentiates "butyrics" and "butyls". *Cl. butylicum* in corn mash medium is a "butyric" type, whereas the addition of asparagin yeast extract or peptone to the medium results in the formation of large quantities of butyl and isopropyl alcohols with little butyric acid.

In table 2 is shown the effect of asparagin on the yields of "solvents" by the butyric acid-butyl alcohol bacteria. Tatum, Peterson and Fred (1935) have shown this effect of asparagin on the "butyric acid forms". It is apparently limited to *Clostridium butylicum* inasmuch as we have never observed the effect on any other than a butyl alcohol-isopropyl alcohol producing culture.

TABLE 3. *The effect of increasing the concentration of asparagin upon the yield of "solvents"*

Species	Culture number	Grams of asparagin added to 300 cc. of corn mash	Percentage of "solvents"
Cl. butylicum	2D	.000	.00
"	2D	.050	0.53
"	2D	.100	1.07
"	2D	.200	9.00
"	2D	.300	16.60
"	2D	.400	20.97
"	53	.000	.00
"	53	.050	1.88
"	53	.100	9.67
"	53	.200	14.52
"	53	.300	18.83
"	53	.400	20.44
"	53	.500	20.44
"	53	.700	20.70

The data show that cultures 3D, 4D, 6D, 12C and 10C, which may be considered as essentially butyric acid types, fail to produce "solvents" in corn mash and are not stimulated to do so by the presence of asparagin. Cultures 2D, 1, 3, 6, 25, 43 and 53 do not produce alcohols in corn mash alone, but in the presence of asparagin, large yields of butyl and isopropyl alcohols occur. Acetone is not present.

That the asparagin effect is quantitative is shown by table 3. A series of flasks containing 5 per cent corn mash (15 gm. per 300 cc.) and increasing quantities of asparagin was inoculated with *Cl. butylicum.* One flask containing no asparagin served as a control. The yield of solvents increases progressively with increase in concentration of asparagin to 0.4 gm. per 300 cc. of medium (0.13 per cent).

Inasmuch as yeast protein in suitable concentration appears to be substantially as stimulating as asparagin (table 4), it was considered prob-

TABLE 4. *Alcohols from corn mash plus asparagin or dried yeast*

Culture number	Corn mash plus 0.1 per cent asparagin			Corn mash plus 1 per cent dried yeast		
	Percentage butyl	Percentage isopropyl	Percentage acetone	Percentage butyl	Percentage isopropyl	Percentage acetone
Cl. butylicum						
2D	13.50	9.10	0.00	12.40	8.53	0.00
53	16.26	6.40	0.00	15.33	5.80	0.00
25	17.16	8.66	0.00	14.98	6.00	0.00

able that the stimulating effect might not be linked with a specific type of amino acid. It is recognized that asparagin does not occur in appreciable amounts in yeast protein, or at least in concentrations necessary to produce marked stimulation. It appears desirable to distinguish between the so-called growth stimulants or metabolic stimulants which have been reported to be highly specific for certain organisms, and nitrogenous constituents. The normal course of fermentation of a given species may be strikingly modified by the absence of more readily available nutrients, although development and abnormal dissimilation may occur.

In view of the above considerations, a variety of nitrogenous substances has been tested regarding their influence on the dissimilation of corn mash. The usual procedure of adding the test substance to 300 cc. of 5 per cent corn mash has been followed throughout. The cultures tested were mainly the isopropyl alcohol-producing organisms and in addition those cultures which failed to produce "solvents" in the presence of asparagin. The mono-amino acids from zein were prepared by acid hydrolysis and extraction with butyl alcohol according to the method of Dakin (1920). The synthetic mixture of mono-amino acids was composed of the acids

TABLE 5. *The effect of yeast and potato extracts, asparagin and peptone on the production of "solvents" in corn mash*

Species	Culture number	Percentage "solvents" in 300 cc. of corn mash plus different nitrogenous substances					
		Corn mash control	1.5 gm. yeast extract	60 cc. crude potato extract	0.400 gm. asparagin	1.5 gm. peptone	5 gm. dried yeast
Cl. butylicum	53	0.78	22.62	13.26	18.72	19.11	23.01
Cl. saccharobutyricum	3D	0.78	0.78	0.78	0.78	0.39	0.78
Cl. Beijerinckii	4D	0.78	1.56	1.56	1.56	0.39	1.56
Cl. Pasteurianum	6D	0.78	3.90	1.56	1.95	1.17	1.95
Cl. Pasteurianum (861)	12C	0.39	1.95	0.78	1.17	0.39	1.95
Cl. pectinovorum (859)	10C	0.78	1.56	0.39	0.39	0.39	0.39
Cl. acetobutylicum (I)	2C	21.06	22.17	21.84	25.22		22.17
Cl. acetobutylicum	101	24.96	22.59	18.72	19.80		22.59
Cl. felsineum	5D	21.45	20.98	17.53	27.30		22.59

TABLE 6. *The influence of certain nitrogenous substances on the production of "solvents" in corn mash*

Substances added to 300 cc. of 5 per cent corn mash	Percentage of "solvents" Culture No. 25	Percentage of "solvents" Culture No. 53
Corn mash control	0.80	0.54
Asparagin, 300 mgm.	19.90	17.75
Aspartic acid, 300 mgm.	....	6.99
Adenine, 300 mgm.	....	11.29
Cystine, 300 mgm.	3.76	1.34
Glycine, 300 mgm.	2.69	1.61
Phenylalanine, 300 mgm.	....	1.07
Arginine carbonate	7.26	1.07
Uracil, 300 mgm.	5.38	1.07
Urea, 300 mgm.	10.22	3.23
Tyrosine, 300 mgm.	2.15	1.61
Mono-amino acids from zein, 300 mgm.	16.14	14.52
Dried yeast, 5 grams	20.98	21.52
Yeast extract (powder), 2 grams	19.90	20.44
Ammonium sulphate, 300 mgm.	....	9.36
Cystine, 300 mgm. plus $(NH_4)_2SO_4$, 300 mgm.	....	10.92
Glutathion, 25 mgm.	1.56	....
Mono-amino acids from zein, 300 mgm. plus $(NH_4)_4SO_2$	14.82	14.04
Mono-amino acids, (synthetic mix)	8.19	9.36

present in zein in approximately the proportion in which they occur. The results are presented in tables 5 and 6.

The data presented in table 5 show that *Cl. butylicum* 53, the isopropyl alcohol-producing organism, was markedly stimulated by asparagin, potato extract, yeast protein and peptone. There was no appreciable effect noted with the remaining species of *Cl. butylicum* 2D, *Cl. Beijerinckii* 4D, *Cl. Pasteurianum* 6D, *Cl. Pasteurianum* 12C (861), and *Cl. pectinovorum* 10C (859), with any of the nitrogenous substances used. The yields of "solvents" produced by the butyl-acetone organisms were practically the same with and without the added nitrogen.

A variety of nitrogen-containing substances including ammonium sulphate and urea (table 6) appear to exert some influence on the yields of alcohols from the fermentation of corn mash by isopropyl alcohol-producing organisms.

SUMMARY

The butyric acid-butyl alcohol group of bacteria may be subdivided into two sections on the basis of their behavior toward acetylmethylcarbinol: (1) those forming the carbinol but failing to reduce it to 2,3-butylene glycol, *Clostridium acetobutylicum* and *Clostridium felsineum*, and (2) those reducing added carbinol to 2,3-butylene glycol.

Addition of asparagin to a corn mash medium diverts the fermentation to form large yields of butyl alcohol in place of butyric acid. Yeast extract and peptone exerted a similar effect.

The effect of asparagin proved to be proportional to its concentration and is apparently specific for *Cl. butylicum* since it was not observed with any but the isopropyl alcohol-forming organism.

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AN INTERESTING TEXAS CUCURBIT

CUCURBITA PEPO, L. VAR OVIFERA ALEF. (C. TEXANA, GRAY)[1]

A. T. ERWIN

From the Vegetable Crops Subsection, Iowa Agricultural Experiment Station

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More than a century ago, Berlandier, an intrepid Swiss botanical explorer and protege of the elder DeCandolle, journeyed from Europe to old Mexico, where he later joined the staff of the United States-Mexican Boundary Commission. In 1835, while botanizing in the region, which later became the state of Texas, he collected a species of cucurbit. A decade later, Jacob Lindheimer, a German botanist, observed the same plant and noted that it was "apparently indigenous to this region," but Asa Gray, who made a critical study of the Plantae Lindheimerianae[2], stated that the "pyriform fruit is just that of *Cucurbita ovifera,* of which our plant may possibly be only a naturalized variety." This opinion was shared by Coulter, who stated, "The common pumpkin has a naturalized variety in southern and western Texas, the habitat, foliage and fruit of which is too well known to need a description." Viewed, therefore, as a garden escape this plant was of no special interest to either the botanist or horticulturist.

A century later, fragments of cucurbit material were recovered from archeological explorations made in the southwest. Many of these specimens were found to be in an excellent state of preservation despite their age, some of them being very ancient, coming from the basket-makers, a culture antedating the cliff-dwellers, the most ancient agricultural peoples on the North American continent of whom we have any knowledge. Among these fragments were found specimens of rind, pedicel and seed, identified by the writer[3] as *Cucurbita pepo,* L. The archeologists have also brought to light historic specimens of pottery fashioned after forms of this same species of cucurbit. Many of these faithfully depict in detail the shape and pedicel markings of the forms from which they were modeled. Thus we now have three important sources of evidence from the same general region as to the nativity of this plant. This information leads to the question—is this Texas cucurbit a garden escape as suggested by Gray, or is it the proto-type from whence came the numerous cultivated forms of *Cucurbita pepo?* Is it an indigene or a foreigner?

An auto trip through the southwest in 1934 and again in 1936 afforded the writer his long sought opportunity to observe this plant in the wild. Through the courtesy of Professor B. C. Tharp of the University of Texas, it was located in Travis County, Texas, on the flood plain of the Colorado River, where it grows luxuriantly in the thickets. The environs strongly indicate that it is an indigenous plant and the surroundings do not suggest that of a garden escape. Bailey, who recently "overtook this plant in

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[1] Journal paper No. J-492 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 298.

[2] Boston Journal of Botany. 1850. 7:(11), Art. 1.

[3] Erwin, A. T., 1931. "Nativity of the Cucurbits," Bot. Gazette, XCI (1).

the wild," observes that it is "apparently indigenous in river valleys of southern Texas;" and Tharp of the University of Texas, who has observed this plant for several years and collected it in various localities, thinks there is no doubt as to its being a native plant.

Berlandier found it along the San Fernando River (Plate III); Lindheimer's specimens came from the upper Guadaloupe; Tharp found it on the Edwards Plateau; Bailey reports it from the lower Colorado River; but Castetter writes the author that although he has been on the lookout for this species in New Mexico, he has never encountered it. Although from its ecology this species might occur in the state of Chihuahua, Mexico, yet we never found it in our by-no-means thorough searches in the winter of 1935; nor did we discover specimens from this region in the herbarium of the Instituto de Biologia of the University of Mexico, though Miss Bravo, of the botany staff of that institution, believes that it is native to this state and that a more diligent search would be quite worthwhile.

Botanically, this Texas cucurbit has had a varied career. It was first described by Scheele under the names *Tristemon texana*, gen, et. sp. nov., then transferred to the genus Cucurbita by Gray; and later the species texana was determined to be a form of pepo.

Cucurbita pepo is a multifarious species and embraces a number of diverse types, one of which is the group of ornamental yellow-flowered garden gourds, designated as variety ovifera. It is to this variety, the least removed from the feral type, that the Texas plant is associated.

We have grown the Texas plant in the greenhouse at Iowa State College and also in the garden, and with the exception of certain minor characters, to which we shall refer later, it is indistinguishable from ovifera (Plate II, fig. A). In flower, leaf, fruit and seed, texana shares essential characteristics with the ornamental gourds, clearly belongs to the same botanical variety as ovifera, and, on the basis of priority, should become a synonym of that variety.

One of the minor variations between *C. texana* and the garden gourds is in the calyx. The sepals of the Texas form are branched near the tip, a character we have not observed in the garden oviferas. The leaves of a young plant are entire, while on the older parts of the vine they are often deeply incised with lobes similar to those of the ornamental gourds. The spicules are apparently less pronounced than in the cultivated oviferas. Judging from our greenhouse plants this species is a short-day plant, since they have flowered freely in the months of November and December at Ames.

If we accept texana as being a form of *Cucurbita pepo* variety *ovifera*, then logically we must assume that the cultivated forms of ovifera came from texana rather than texana is a naturalized form of the latter. The plasticity of the oviferas is shown in Bailey's interesting book on "The Garden of Gourds," and this variety embraces a multiplicity of variations in form and color.

Texana readily responds to culture. The greenhouse plants produce leaves more than double the size of those of the wild. They flower freely and pistillate flowers appear rather early in the reproductive phase, which is unusual in most of the cucurbits. In view of these facts, it does not call for any stretching of the imagination to assume that other variations, such as a thickening of the rind and the elimination of the bitter taste, also, may

have occurred to this Texas plant, giving us our edible as well as ornamental forms of *Cucurbita pepo.*

Linnaeus based his description of *Cucurbita pepo* upon specimens grown under cultivation, nativity not designated. The fact may also be noted that this plant was unknown to the old world in pre-Columbian times. Its introduction into Europe is readily accounted for by the Spanish Fathers who sent back to the old world seed of numerous plants new to them.

It is significant that *Cucurbita pepo* existed in the southwest ages ago, as attested by archeological evidence, and that the Texas form of the same species has survived in the wild over a considerable range in this same region for over a century. Is texana to be accepted as the prototype of our cultivated forms of *Cucurbita pepo?* We think the preponderance of the evidence, to date, is to that effect.

PLATE I

Cucurbita pepo L. var. ovifera Alef. (C. texana Gray)

Figs. A, 13, C—Pistillate flowers (3 x).
Fig. D—Tendril.
Fig. E—Pollen grain (250 x).
Fig.s F, G—Staminate flowers (3 x).
Fig. H—Leaf of young plant.
Fig. I—Stem (10 x).

Drawings by Miss Marie A. Corkle

PLATE I

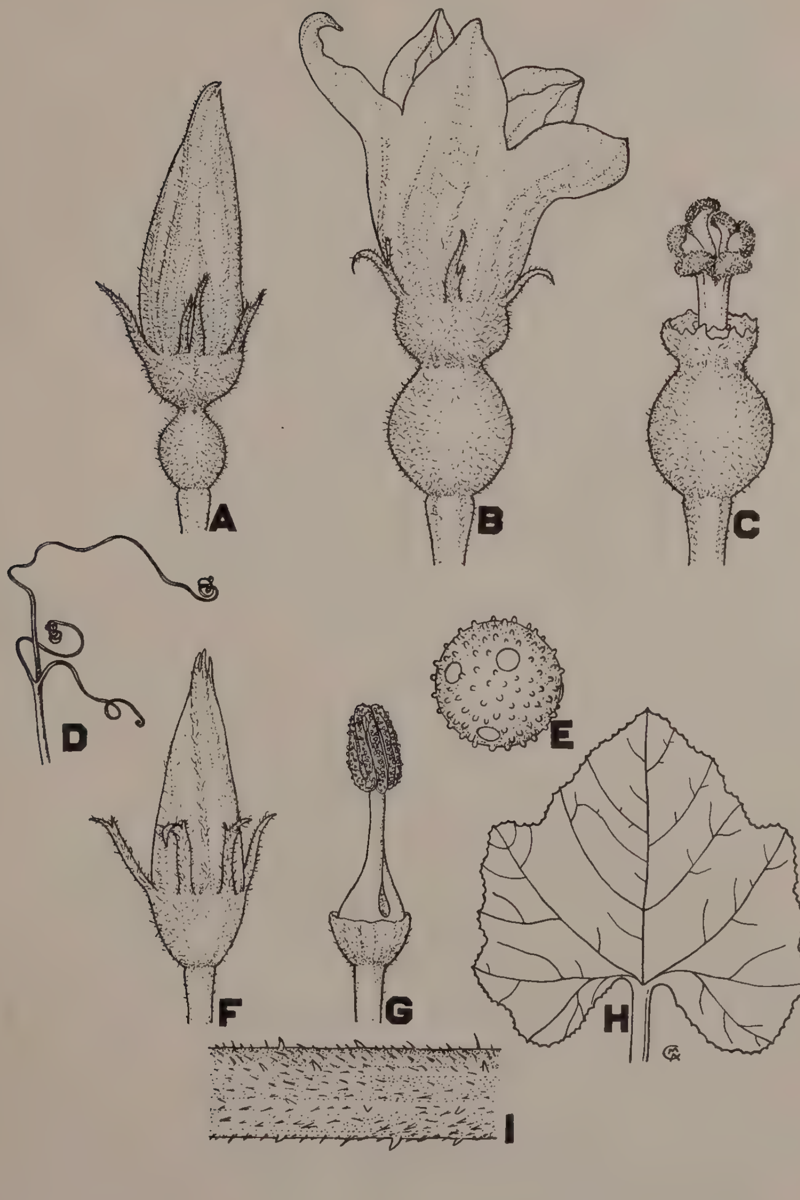

PLATE II

Cucurbita pepo L. var. ovifera Alef. (C. texana Gray)

Fig. A—Plant grown in greenhouse.

Fig. B—Typical fruit from a wild plant. The fruits vary in form from pyriform to globular.

PLATE II

PLATE III

Cucurbita pepo L. var. ovifera Alef. (C. texana Gray)

Berlandier specimen from the Gray Herbarium, Courtesy of Dr. M. L. Fernald.

PLATE III


GRAY HERBARIUM
HARVARD
UNIVERSITY
Herbarium Berlandierianum Texano-Mexicanum

ZEROS OF THE LEGENDRE POLYNOMIALS

E. R. Smith

From the Department of Mathematics, Iowa State College

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In this paper two formulas which may be used to compute approximate values for the zeros of the Legendre Polynomials are derived. One of the formulas is intended primarily for the values near zero and the other for the values near unity. Either of the two formulas is suitable for computing the middle values.

The method by which the formulas have been obtained is an adaptation of the one which was used by Zernike[1] in obtaining the largest zero of the Hermitian polynomials. A table giving the zeros, to six decimal places, up to $n = 40$ is included.

The small zeros. The polynomials of Legendre are solutions of the differential equation,

$$(1) \qquad (1-x^2)P_n''(x) - 2x\,P_n'(x) + n(n+1)P_n(x) = 0,$$

in which n takes on successive positive integral values. If $x = \sqrt{\lambda}\ t$, where $\lambda = \dfrac{1}{n(n+1)}$, is substituted in the equation it is changed to

$$(2) \qquad (1-\lambda t^2)P_n'' - 2\lambda t P_n' + P_n = 0.$$

The solutions of this equation which correspond to the polynomials of Legendre are, for n an even integer,

$$(3) \qquad 1 - \frac{t^2}{2!} + (1-2\cdot 3\lambda)\frac{t^4}{4!} - (1-2\cdot 3\lambda)(1-4\cdot 5\lambda)\frac{t^6}{6!} + \ . \ . \ . \ ,$$

and for n an odd integer,

$$(4) \qquad t - (1-1\cdot 2\lambda)\frac{t^3}{3!} + (1-1\cdot 2\lambda)(1-3\cdot 4\lambda)\frac{t^5}{5!} - \ . \ . \ . \ .$$

It is readily seen that (3) and (4) have the same zeros as the polynomials of Legendre.

Let

$$(5) \qquad P_n = \omega_0 + \omega_1\lambda + \omega_2\lambda^2 + \omega_3\lambda^3 + \ . \ . \ .$$

where $\omega_0, \omega_1, \omega_2, \omega_3, \ . \ . \ .$ are functions of t which are independent of the parameter λ. We substitute (5) in (2) and obtain

[1] Zernike, Eine asymptotic Entwickelung für die grösste Nullstelle der Hermiteschen Polynome, Proc. K. Akademie van Wetenschappen Amsterdam, 34:673. (1931).

$$(1-\lambda t^2)(\omega_0'' + \omega_1''\lambda + \omega_2''\lambda^2 + \ . \ . \ . \ )$$
$$- 2\lambda t(\omega_0' + \omega_1'\lambda + \omega_2'\lambda^2 + \ . \ . \ . \ )$$
$$+ (\omega_0 + \omega_1\lambda + \omega_2\lambda^2 + \ . \ . \ . \ ) = 0.$$

Hence

$$\omega_0'' + \omega_0 = 0,$$

$$\omega_1'' + \omega_1 = \frac{d}{dt}(t^2\omega_0'), \tag{6}$$

$$\omega_2'' + \omega_2 = \frac{d}{dt}(t^2\omega_1'),$$

$$. \ . \ . \ . \ . \ . \ . \ . \ . \ . \ ,$$

$$\omega_n'' + \omega_n = \frac{d}{dt}(t^2\omega_{n-1}),$$

$$. \ . \ . \ . \ . \ . \ . \ . \ . \ . \ .$$

If the subscript of P_n is even the solutions of these equations must satisfy the following initial conditions:

$$\text{for } t=0, \quad \omega_0 = 1 \quad \text{and} \quad \omega_n = 0,$$
$$\omega_0' = 0 \quad \text{and} \quad \omega_n' = 0.$$

If the subscript of P_n is odd then

$$\text{for } t=0, \quad \omega_0 = 0 \quad \text{and} \quad \omega_n = 0,$$
$$\omega_0' = 1 \quad \text{and} \quad \omega_n' = 0.$$

To solve the system of equations (6) we let

$$\omega_n = p_n\omega_0 + q_n\omega_0'.$$

By substituting this expression in (2) we may obtain recurrence relations for the determination of p_n and q_n. It follows that

$$(p_n'' - 2q_n')\omega_0 + (q_n'' + 2p_n')\omega_0' =$$
$$[t^2(p''_{n-1} - 2q'_{n-1} - p_{n-1}) + 2t(p'_{n-1} - q_{n-1})]\omega_0$$
$$+ [t^2(q''_{n-1} + 2p'_{n-1} - q_{n-1}) + 2t(q'_{n-1} + p_{n-1})]\omega_0'.$$

Hence

$$p_n'' + 2q_n' = t^2(p''_{n-1} - 2q'_{n-1} - p_{n-1}) + 2t(p'_{n-1} - q_{n-1}),$$
$$q_n'' + 2p_n' = t^2(q''_{n-1} + 2p'_{n-1} - q_{n-1}) + 2t(q'_{n-1} + p_{n-1}).$$

The general solution for ω_0 may be written as

$$\omega_0 = p_0\omega_0 + q_0\omega_0',$$

where p_0 and q_0 are constants. Hence the initial conditions require that $p_0 = 1$ and $q_0 = 0$. Using these values the foregoing equations may be successively solved for p_n and q_n so as to satisfy the initial conditions.

For n an even integer we obtain

$$p_0 = 1,$$

$$q_0 = 0,$$

$$p_1 = \frac{t^2}{4},$$

$$q_1 = \frac{t^3}{6} + \frac{t}{4},$$

$$p_2 = -\frac{1}{72} t^6 + \frac{11}{96} t^4 - \frac{3}{32} t^2,$$

$$q_2 = \frac{7}{60} t^5 + \frac{7}{48} t^3 - \frac{3}{32} t,$$

$$p_3 = -\frac{23}{1440} t^8 + \frac{427}{5760} t^6 - \frac{35}{384} t^4 + \frac{23}{128} t^2,$$

$$q_3 = -\frac{1}{1296} t^9 + \frac{1733}{20160} t^7 + \frac{193}{1820} t^5 - \frac{29}{192} t^3 + \frac{23}{128} t,$$

$$p_4 = \frac{1}{31104} t^{12} - \frac{55721}{3628800} t^{10} + \frac{35521}{645120} t^8 - \frac{3829}{46080} t^6 + \frac{1917}{6144} t^4 - \frac{1549}{2048} t^2,$$

$$q_4 = -\frac{1}{810} t^{11} + \frac{4091}{60480} t^9 + \frac{12499}{161280} t^7 - \frac{873}{5120} t^5 + \frac{1733}{3072} t^3 - \frac{1549}{2048} t,$$

$$p_5 = \frac{41}{622080} t^{14} - \frac{617603}{43545600} t^{12} + \frac{565913}{12902400} t^{10} - \frac{32659}{430080} t^8 + \frac{30125}{73728} t^6$$
$$- \frac{50875}{24576} t^4 + \frac{44679}{8192} t^2,$$

$$q_5 = \frac{1}{933120} t^{15} - \frac{15913}{10886400} t^{13} + \frac{438981}{7884800} t^{11} + \frac{163397}{2580480} t^9 - \frac{15283}{68016} t^7$$
$$+ \frac{120107}{122880} t^5 - \frac{47777}{12288} t^3 + \frac{44679}{8192} t,$$

. . . .

For n an odd integer the corresponding solutions are

$$p_0 = 1,$$

$$q_0 = 0,$$

$$p_1 = \frac{1}{4} t^2 - \frac{1}{4},$$

$$q_1 = \frac{1}{6} t^3 + \frac{1}{4} t,$$

$$p_2 = -\frac{1}{72} t^6 + \frac{11}{96} t^4 - \frac{5}{32} t^2 + \frac{5}{32},$$

$$q_2 = \frac{7}{60} t^5 + \frac{5}{48} t^3 - \frac{5}{32} t,$$

$$p_3 = -\frac{23}{1440} t^8 + \frac{149}{1920} t^6 - \frac{23}{192} t^4 + \frac{31}{128} t^2 - \frac{31}{128},$$

$$q_3 = -\frac{1}{1296} t^9 + \frac{1733}{20160} t^7 + \frac{137}{1920} t^5 - \frac{31}{192} t^3 + \frac{31}{128} t,$$

$$p_4 = \frac{1}{31104} t^{12} - \quad . \quad . \quad . \quad ,$$

$$q_4 = -\frac{1}{810} t^{11} + \frac{3077}{45360} t^9 + \frac{3011}{53760} t^7 - \frac{545}{3072} t^5 + \frac{1795}{3072} t^3 - \frac{1795}{2048} t,$$

$$p_5 = \frac{41}{622080} t^{14} - \quad . \quad . \quad . \quad ,$$

$$q_5 = \frac{1}{933120} t^{13} - \quad . \quad . \quad . \quad ,$$

. . . .

Let α be a zero of ω_0. It is evident that for n even $\alpha = \frac{2k+1}{2}\pi$ and for n odd $\alpha = k\pi$, where $k = 0, 1, 2, 3, \ldots$.

We now assume that the zeros of P_n are $\alpha + h$ and let

(7) $$h = C_1\lambda + C_2\lambda^2 + C_3\lambda^3 + \quad . \quad . \quad . \quad ,$$

where $C_1, C_2, C_3, \ldots$ are constants independent of λ. Then

(8) $$P(\alpha + h) = P_n(\alpha) + P_n'(\alpha) h + P_n''(\alpha)\frac{h^2}{2!} + \quad . \quad . \quad . \quad = 0.$$

If (5) and (7) are substituted in (8) an identity is obtained in which the coefficients of the successive powers of λ vanish. In this manner a system of equations are obtained for the determination of the constants $C_1, C_2, C_3, \ldots$. We obtain

$$\begin{aligned}
&(\omega_0 + \omega_1\lambda + \omega_2\lambda^2 + \omega_3\lambda^3 + \ldots) \\
&+ (C_1\lambda + C_2\lambda^2 + C_3\lambda^3 + \ldots)(\omega_0' + \omega_1'\lambda + \omega_2'\lambda^2 + \omega_3'\lambda^3 + \ldots) \\
&+ \frac{1}{2}(C_1\lambda + C_2\lambda^2 + C_3\lambda^3 + \ldots)^2(\omega_0'' + \omega_1''\lambda + \omega_2''\lambda^2 + \omega_3''\lambda^3 + \ldots) \\
&+ \frac{1}{6}(C_1\lambda + C_2\lambda^2 + \ldots)^3(\omega_0''' + \omega_1'''\lambda + \omega_2'''\lambda^2 + \ldots) \\
&+ \frac{1}{24}(C_1\lambda + \ldots)^4(\omega_0^{iv} + \omega_1^{iv}\lambda + \ldots) \\
&+ \ldots\ldots = 0.
\end{aligned}$$

The vanishing of the coefficients of the powers of λ give the following equations:

$$\begin{aligned}
&\omega_0 = 0, \\
(9)\qquad &\omega_1 + C_1\omega_0' = 0, \\
&\omega_2 + C_1\omega_1' + C_2\omega_3' + \frac{1}{2}C_1^2\omega_0'' = 0, \\
&\omega_3 + C_1\omega_2' + C_1\omega_1' + C_3\omega_0' + \frac{1}{2}C_1^2\omega_1'' + C_1C_2\omega_0'' + \frac{1}{6}C_1^3\omega_0''' = 0, \\
&\ldots\ldots\ldots\ldots
\end{aligned}$$

We may show that for $t = a$ and n an even integer we may substitute the following or their corresponding negative values in (9):

$$\begin{aligned}
&\omega_0 = 0, && \omega_1 = -\frac{a^3}{6} - \frac{a}{4}, \\
&\omega_0' = -1, && \omega_1' = -\frac{3}{4}a^2 - \frac{1}{4}, \\
&\omega_0'' = 0, && \omega_1'' = \frac{1}{6}a^3 - \frac{7}{4}a, \\
&\omega_0''' = 1, && \omega_1''' = \frac{7}{4}a^2 - \frac{7}{4}, \\
&\omega_0^{iv} = 0, && \omega_1^{iv} = -\frac{1}{6}a^2 + \frac{31}{4}a, \\
&\omega_0^{v} = -1, && \ldots\ldots, \\
&\ldots\ldots
\end{aligned}$$

$$\omega_2 = \frac{7}{60} a^5 - \frac{7}{48} a^3 + \frac{3}{32} a,$$

$$\omega_2' = \frac{1}{72} a^6 - \frac{67}{96} a^4 - \frac{11}{32} a^2 + \frac{3}{32},$$

$$\omega_2'' = \frac{17}{60} a^5 - \frac{149}{48} a^3 - \frac{19}{32} a,$$

$$\omega_2''' = -\frac{1}{72} a^6 + \frac{299}{96} a^4 - \frac{317}{32} a^2 - \frac{19}{32},$$

$$\cdots\cdots\cdots,$$

$$\omega_3 = \frac{1}{1296} a^9 - \frac{1733}{20160} a^7 - \frac{193}{1920} a^5 + \frac{29}{192} a^3 - \frac{23}{128} a,$$

$$\omega_3' = \frac{11}{480} a^8 - \frac{3893}{5760} a^6 - \frac{79}{192} a^4 + \frac{35}{128} a^2 - \frac{23}{128},$$

$$\omega_3'' = \frac{1}{1296} a^9 + \frac{1601}{4032} a^7 - \frac{8447}{1920} a^5 - \frac{275}{192} a^3 + \frac{47}{128} a,$$

$$\cdots\cdots\cdots,$$

$$\omega_4 = \frac{1}{810} a^{11} - \frac{4091}{60480} a^9 + \frac{2499}{161280} a^7 + \cdots,$$

$$\omega_4' = -\frac{1}{31104} a^{12} + \frac{105001}{3628800} a^{10} - \frac{428257}{645120} a^8 + \cdots,$$

$$\cdots\cdots\cdots,$$

$$\omega_5 = -\frac{1}{933120} a^{15} + \frac{15913}{10886400} a^{13} - \frac{43891}{7884800} a^{11} + \cdots,$$

$$\cdots\cdots\cdots$$

When these values have been substituted in the equations (9) the following solutions may be obtained:

$$C_1 = -\frac{a^3}{6} - \frac{a}{4},$$

$$C_2 = \frac{1}{120} a^5 + \frac{1}{12} a^8 + \frac{5}{32} a,$$

$$C_3 = -\frac{1}{5040} a^7 - \frac{1}{160} a^5 + \frac{5}{192} a^3 - \frac{31}{128} a,$$

$$C_4 = \frac{1}{362880}a^9 + \frac{1}{5040}a^7 + \frac{31}{768}a^5 - \frac{53}{128}a^3 + \frac{1795}{2048}a,$$

$$C_5 = -\frac{1}{11!}a^{11} - \frac{5}{4}\frac{1}{9!}a^9 - \frac{3211}{161280}a^7 - \frac{7399}{15360}a^5$$

$$+\frac{42337}{12288}a^3 - \frac{48439}{8192}a,$$

. . . .

It may be shown that the same results are obtained for $C_1, C_2, C_3, \ldots$ when n is an odd integer. We then write for the zeros of $P_n(t)$ the following approximation formula:

$$\begin{aligned}
\text{zero} &= a - \left(\frac{a^3}{3!} + \frac{1}{4}a\right)\lambda \\
&+ \left(\frac{1}{5!}a^5 + \frac{2}{4}\cdot\frac{1}{3}a^3 + \frac{5}{32}a\right)\lambda^2 \\
&- \left(\frac{1}{7!}a^7 + \frac{3}{4}\cdot\frac{1}{5!}a^5 - \frac{5}{192}a^3 + \frac{31}{128}a\right)\lambda^3 \\
&+ \left(\frac{1}{9!}a^9 + \frac{4}{4}\cdot\frac{1}{7!}a^7 + \frac{31}{768}a^5 - \frac{53}{128}a^3 + \frac{1795}{2048}a\right)\lambda^4 \\
&- \left(\frac{1}{11!}a^{11} + \frac{5}{4}\cdot\frac{1}{9!}a^9 + \frac{3211}{161280}a^7 + \ldots\right)\lambda^5 \\
&+ \ldots\ldots\ldots
\end{aligned} \tag{10}$$

Since $x = \sqrt{\lambda}\, t$, the corresponding values of x which are the zeros of $P_n(x)$ are obtained at once by multiplying by $\sqrt{\lambda}$ When this is done the following formula is obtained for the approximation of the zeros:

$$\text{zero} = \left(1 - \frac{\lambda}{8}\right)\sin a\sqrt{\lambda} - a\frac{\lambda^{3/2}}{8}\cos a\sqrt{\lambda} + \theta \tag{11}$$

where

$$\begin{aligned}
\theta &= \frac{5}{32}a\lambda^{5/2} \\
&+ \left(\frac{5}{192}a^3 - \frac{31}{128}a\right)\lambda^{7/2} \\
&+ \left(\frac{31}{728}a^5 - \frac{53}{128}a^3 + \frac{1795}{2048}a\right)\lambda^{9/2} \\
&+ \left(\frac{3211}{161280}a^7 - \frac{7399}{15360}a^5 + \ldots\right)\lambda^{11/2} \\
&+ \ldots\ldots
\end{aligned}$$

The zeros near unity. In equation (1) substitute $x = 1 + 2\lambda t$ and obtain:

$$(12) \qquad (t + \lambda t^2) P_n'' + (1 + 2\lambda t) P_n' - P_n = 0.$$

Let

$$(13) \qquad P_n = v_0 + v_1\lambda + v_2\lambda^2 + v_3\lambda^3 + \cdots$$

and obtain from (12)

$$\begin{aligned}&(t + \lambda t^2)(v_0'' + v_1''\lambda + v_2''\lambda^2 + \cdots)\\ &+ (1 + 2\lambda t)(v_0' + v_1'\lambda + v_2'\lambda^2 + \cdots)\\ &- (v_0 + v_1\lambda + v_2\lambda^2 + \cdots) = 0.\end{aligned}$$

From this identity we set up the following system of differential equations for the determination of $v_0, v_1, v_2, v_3, \ldots$.

$$\begin{aligned}&tv_0'' + v_0' - v_0 = 0,\\ &tv_1'' + v_1' - v_1 = -\frac{d}{dt}(t^2 v_0'),\\ &tv_2'' + v_2' - v_2 = -\frac{d}{dt}(t^2 v_1'),\\ &\cdots\cdots\cdots\cdots,\\ &tv_n'' + v_n' - v_n = -\frac{d}{dt}(t^2 v'_{n-1}),\\ &\cdots\cdots\cdots\cdots\end{aligned}$$

A particular solution for the first of these differential equations is seen to be in the form of a Bessel's function

$$(14) \qquad v_0 = J_0(2i\sqrt{t}\,) = 1 + t + \frac{t^2}{[2!]^2} + \frac{t^3}{[3!]^2} + \cdots.$$

To obtain the solution for v_n $(n > 0)$ we assume that

$$v_n = r_n v_0 + s_n v_0'$$

where r_n and s_n are functions of t. Substitutions give the following recurrence relations:

$$\begin{aligned}&t^2r_n'' + tr_n' + 2ts_n' - s_n = -t^3r''_{n-1} - 2t^2r'_{n-1} - t^2r_{n-1} - 2t^2s'_{n-1}\\ &2t^2r_n' + t^2s_n'' - ts_n' + s_n = -2t^3r'_{n-1} - t^2r_{n-1} - t^3s''_{n-1} - t^2s_{n-1}.\end{aligned}$$

Solution for the successive expressions for r_n and s_n gives

$$r_1 = -\frac{1}{3}t,$$

$$s_1 = \frac{1}{3}t - \frac{1}{3}t^2,$$

$$r_2 = \frac{1}{15}t + \frac{1}{10}t^2 + \frac{1}{18}t^3,$$

$$s_2 = -\frac{1}{15}t - \frac{2}{15}t^2 + \frac{7}{30}t^3,$$

$$r_3 = -\frac{8}{315}t - \frac{4}{105}t^2 - \frac{103}{1890}t^3 - \frac{53}{810}t^4,$$

$$s_3 = \frac{8}{315}t + \frac{16}{315}t^2 + \frac{1}{14}t^3 - \frac{907}{5670}t^4 - \frac{1}{162}t^5,$$

$$r_4 = \cdot\ \cdot\ \cdot\ \cdot\ \cdot\ \cdot\ \cdot\ \cdot\ ,$$
$$s_4 = \cdot\ \cdot\ \cdot\ \cdot\ \cdot\ \cdot\ \cdot\ \cdot\ ,$$

. . . .

Let β be a zero of v_0, and assume that the zeros of the transformed polynomial are of the form

$$\beta + b_1\lambda + b_2\lambda^2 + b_3\lambda^3 + \ .\ .\ .\ .$$

We may obtain an expression for the zeros in a manner similar to that which has already been used. Computation gives

$$b_1 = -\frac{1}{3}\beta + \frac{1}{3}\beta^2,$$

$$b_2 = \frac{11}{90}\beta - \frac{1}{5}\beta^2 + \frac{2}{45}\beta^3,$$

$$b_3 = -\frac{61}{1134}\beta + \frac{241}{2835}\beta^2 - \frac{4}{105}\beta^3 + \frac{1}{135}\beta^4,$$

$$b_4 = \cdot\ \cdot\ \cdot\ \cdot\ \cdot\ \cdot\ \cdot\ \cdot\ ,$$

. . . .

We now return to the original variable

$$x = 1 + 2\lambda t$$

and write for the values of x which are zeros of the polynomials of Legendre the following expression:

$$\text{zero} = 1 + 2\beta\lambda - \frac{2}{3}(\beta - \beta^3)\lambda^2 + \frac{1}{45}(11\beta - 18\beta^2 + 4\beta^3)\lambda^3$$

$$-\frac{1}{2835}(305\beta - 482\beta^2 + 216\beta^3 - 42\beta^4)\lambda^4 + \ .\ .\ . \qquad (15)$$

The values of β which are zeros of Bessel's function may be taken from existing tables[2]. We obtain directly the value of $2i\sqrt{\beta}$, from which β may be obtained. Thus if the successive zeros are $\beta_1, \beta_2, \beta_3, \ldots$ in ascending order of magnitudes

$$\begin{aligned}
\beta_1 &= -\ \ 1.4457965 \\
\beta_2 &= -\ \ 7.6178156 \\
\beta_3 &= -18.7217516 \\
\beta_4 &= -34.7603069 \\
\beta_5 &= -55.7330759 \\
\beta_6 &= -81.6408382 \\
&\ldots\ldots\ldots\ldots
\end{aligned}$$

In computing the zeros of the Legendre Polynomials, actual work has shown that beginning with the smallest value about two-thirds should be computed using formula (10) and the remainder using formula (15).

A table of the zeros of the Legendre Polynomials up to $n = 40$ is given here. The values up to and including $n = 7$ were computed by three British graduate students in 1873 and have been taken from the Report of the British Association for the Advancement of Science, 1879, pp. 49-57.

The zeros from $n = 7$ to $n = 25$ were computed in 1934 by Dr. Archie Higdon. He used a method based on some theorems by Sturm similar to the method used by the author in the computation of the zeros of the Hermitian polynomials[3]. The remainder of the zeros were computed using the formulas of this paper.

For each value of n the values have been checked by comparing the sum of their squares with $\dfrac{n(n-1)}{2(2n-1)}$.

POSITIVE ZEROS OF THE LEGENDRE POLYNOMIALS

n = 1	n = 2	n = 3	n = 4	n = 5
.000000	.577350	.000000	.339981	.000000
		.774597	.861136	.538469
				.906180

n = 6	n = 7	n = 8	n = 9	n = 10
.238619	.000000	.183435	.000000	.148874
.661209	.405845	.525533	.324253	.433395
.932470	.741531	.796666	.613371	.679410
	.949108	.960290	.836031	.865063
			.968160	.973907

[2] Davis, H. T., and W. J. Kirkham. A new table of the zeros of the Bessel functions, Bull. Amer. Math. Soc. 33: 760-772. (1927).

[3] American Math. Monthly, 43: 354-358 (1936).

n = 11	n = 12	n = 13	n = 14	n = 15
.000000	.125233	.000000	.108055	.000000
.269543	.367832	.230458	.319112	.201194
.519095	.587318	.448493	.515249	.394151
.730152	.769903	.642349	.687293	.570972
.887063	.904117	.801578	.827202	.724418
.978229	.981561	.917598	.928435	.848207
		.984183	.986284	.937273
				.987993

n = 16	n = 17	n = 18	n = 19	n = 20
.095012	.000000	.084775	.000000	.076527
.281605	.178484	.251886	.160359	.227786
.458017	.351232	.411751	.316564	.373706
.617876	.512691	.559771	.464571	.510867
.755404	.657671	.691687	.600545	.636054
.865631	.781514	.803705	.720966	.746332
.944575	.880239	.892603	.822715	.839117
.989401	.950676	.955824	.903156	.912235
	.990575	.991565	.960208	.963972
			.992407	.993129

n = 21	n = 22	n = 23	n = 24	n = 25
.000000	.069739	.000000	.064057	.000000
.145562	.207860	.133257	.191119	.122865
.288021	.341936	.264136	.315043	.243867
.424342	.469356	.390301	.433794	.361172
.551619	.587640	.509502	.545421	.473003
.667139	.694487	.619610	.648094	.577663
.768440	.787817	.718661	.740124	.673566
.853363	.865812	.804888	.820002	.759259
.920100	.926957	.876752	.886415	.833443
.967227	.970061	.932971	.938275	.894992
.993752	.994295	.972542	.974729	.942975
		.994769	.995187	.976664
				.995557

n = 26	n = 27	n = 28	n = 29	n = 30
.059230	.000000	.055079	.000000	.051472
.176859	.113973	.164569	.106278	.153870
.292005	.226459	.272062	.211352	.254637
.403052	.335994	.376252	.314032	.352705
.508441	.441148	.475874	.413153	.447034
.606692	.540552	.569720	.507593	.536624
.696427	.632908	.656651	.596282	.620526
.776386	.717013	.735611	.678215	.697850
.845446	.791772	.805641	.752463	.767777
.902638	.856208	.865892	.818185	.829566
.947159	.909482	.915633	.874638	.882560
.978385	.950901	.954259	.921180	.926200
.995886	.979923	.981303	.957286	.960022
	.996179	.996442	.982545	.983668
			.996679	.996893

n = 31	n = 32	n = 33	n = 34	n = 35
.000000	.048308	.000000	.045510	.000000
.099555	.144472	.093631	.136152	.088371
.198121	.239287	.186439	.225667	.176051
.294718	.331869	.277609	.313311	.262353
.388386	.421351	.366339	.398359	.346602
.478194	.506900	.451850	.480106	.428138
.563249	.587716	.533390	.557876	.506323
.642707	.663044	.610242	.631022	.580545
.715777	.732182	.681732	.698939	.650224
.781733	.794484	.747231	.761065	.714814
.839920	.849368	.806162	.816884	.773810
.889760	.896321	.858010	.865935	.826750
.930757	.934906	.902317	.907810	.873219
.962504	.964762	.938694	.942162	.912854
.984686	.985612	.966823	.968708	.945345
.997087	.997264	.986456	.987228	.970438
		.997425	.997572	.987936
				.997707

n = 36	n = 37	n = 38	n = 39	n = 40
.043018	.000000	.040785	.000000	.038772
.128736	.083670	.122084	.079444	.116084
.213501	.166754	.202570	.158385	.192698
.296685	.248668	.281709	.236326	.268152
.377673	.328837	.358972	.312772	.341994
.455864	.406701	.433848	.387240	.413779
.530680	.481711	.505835	.459261	.483076
.601568	.553341	.574456	.528377	.549467
.668001	.621093	.639255	.594153	.612554
.729489	.684486	.699799	.656173	.671957
.785576	.743079	.755686	.714044	.727318
.835847	.796459	.806544	.767401	.778306
.879930	.844253	.852035	.815906	.824612
.917498	.886125	.891856	.859253	.865960
.948273	.921781	.925741	.897167	.902099
.972028	.950972	.953466	.929409	.932813
.988586	.973493	.974846	.955775	.957917
.997830	.989186	.989739	.976099	.977260
	.997945	.998050	.990252	.990726
			.998147	.998238

DISSIMILATION OF INTERMEDIARY COMPOUNDS IN THE BUTYL-ISOPROPYL ALCOHOL FERMENTATION[1]

O. L. Osburn, R. W. Brown and C. H. Werkman

From the Agricultural By-Products Laboratory
United States Department of Agriculture, and the Bacteriology Section,
Iowa Agricultural Experiment Station

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The reactions involved in fermentative processes are often obscured by the highly reactive state of the intermediary molecules occurring during the course of the dissimilation. As shown by the writers in a previous paper (1937), the addition of reagents which partially inhibit some of the enzymatic reactions in the butyl-isopropyl alcohol fermentation, leads to the accumulation of measurable quantities of intermediary products.

This paper deals with the dissimilation of intermediary substances in the butyl alcohol-isopropyl alcohol fermentation *(Clostridium butylicum)* when added to fermenting glucose media and the fermentation is allowed to proceed. Such data serve in formulating the reactions involved in the dissimilation of glucose by *Clostridium butylicum.*

The occurrence of a series of reactions in the dissimilation of carbohydrates by microorganisms seems well established. The butyl alcohol-acetonic fermentation has been the subject of many investigations and a series of reactions has been formulated to account for the end products from glucose. Suggestions that acetaldehyde plays an important role in the formation of 4-carbon compounds have been made by Pasteur (1862), Fitz (1876-82), Buchner and Meisenheimer (1908), and Neuberg and Arinstein (1921). Apparently these suggestions were based, for the most part, on catalytic-organic reactions such as those studied by Schade (1907), $C_6H_{12}O_6 \xrightarrow[\text{KOH}]{\text{Strong}} CH_3CHOHCOOH \xrightarrow{H_2SO_4} CH_3CHO + HCOOH$

$\longrightarrow CH_3CH_2OH + CO_2$; Hoppe-Seyler (1878), who obtained lactic acid by heating glycerol with strong potassium hydroxide solution; Duclaux (1887), who showed that, in the presence of mercuric salts, sunlight converted calcium lactate into butyric acid, and Makowka (1908), who obtained a quantitative yield of butyric acid by heating palladium-acetylene with potassium hydroxide. According to Buchner and Meisenheimer (1908), working with fermentations, this last reaction can proceed only through the intermediate stages of aldol or of crotonaldehyde. Working with *Bacillus butylicus* (Fitz) Buchner and Meisenheimer (1908) obtained the following results:

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100 gms.	Butyl Alc.	Ethyl Alc.	Carbon Diox.	Hydro-gen	Formic acid	Buty-ric acid	Acetic acid	Lactic acid
Glycerol	19.6	10.4	42.1	1.9	4.0	0.7	1.0	3.4
Glucose	0.7	2.8	48.1	1.6	3.4	26.0	7.5	10.0

Basing their conclusions on the end products of these two fermentations, Buchner and Meisenheimer proposed the following reactions:

$$C_6H_{12}O_6 \longrightarrow CH_3CHOHCOOH \longrightarrow 2CH_3CHO + 2\,HCOOH$$

$$2CH_3CHO \longrightarrow CH_3CHOHCH_2CHO \begin{cases} \longrightarrow CH_3CH_2CH_2COOH \\ \longrightarrow CH_3CH_2CH{:}\,CHCHO \\ \qquad\text{(crotonic aldehyde)} \end{cases}$$

$$\xrightarrow{+\,4H} CH_3CH_2CH_2CH_2OH$$

$$HCOOH \longrightarrow CO_2 + H_2$$

Reilly *et al* (1920) and Speakman (1920-23) showed that production of alcohols and acetone (acetone had not been found among fermentation products at the time of Buchner and Meisenheimer) was preceded by formation of butyric and acetic acids. As the neutral products were formed the concentration of the acids decreased. Acetic and butyric acids added to the fermentations were converted respectively into acetone and butyl alcohol.

Donker (1926) and van der Lek (1930) summarized earlier work and proposed reactions for the complete fermentation. The reactions are briefly: glucose is converted into methylglyoxal; methylglyoxal is hydrated and split into formic acid and acetaldehyde; acetic acid is formed by the hydration of acetaldehyde and removal of 2H; acetaldehyde condenses to aldol and is converted into butyric acid and butyl alcohol, as shown by Buchner and Meisenheimer reactions in the foregoing paragraphs; acetone and isopropyl alcohol are formed from acetic acid through the intermediary formation of acetoacetic acid.

The investigation of van der Lek (1930) on the production of butyl and isopropyl alcohols, is the only work of a quantitative nature. Langlykke and Fred (1937) found: (1) the formation of isopropyl alcohol was preceded by accumulation of acetone in solution, (2) the addition of pyruvic acid to fermenting glucose increased the yields of normal fermentation products and (3) acetaldehyde was converted to ethyl alcohol.

In the experimental data, which follows, the cultural and analytical methods are the same as those used in former work (1937).

EXPERIMENTAL

DESCRIPTION OF THE ORGANISM

No complete description of the organism used in this investigation has appeared in the literature. The culture is a transplant of one prepared by Beijerinck (1893) in his laboratory in Delft and later recovered by Prof. Kluyver and used by van der Lek (1930). The tube had lain for

approximately 38 years before the dried spore material was revived. The authors thank Prof. Kluyver for a transplant.

Name: *Clostridium butylicum* (Beijerinck) Donker.

Synonyms: *Granulobacter butylicum* (Beijerinck).

Clostridium americanum (Pringsheim).

Source: Cereal mashes.

Morphology. Medium: Four per cent corn mash containing potato extract. Age of culture, 24 hours. Temperature of incubation, 37° C. Form: rods, 2 to 5 microns long, 0.7 to 1.5 microns in width. Arrangement: single, pairs, chains. Ends: rounded. Spores: present, abundant in old cultures. Granulose in young cells. Zoogloea formed in unfavorable media.

Cultural characteristics: Colony. Malt extract-gelatin agar; deep colonies rounded, white to yellow; surface growth spreading. Optimal temperature 37° C. Obligate anaerobe. Peritrichous flagella. Gram positive in young cultures; may be gram negative in old cultures.

Biochemical reactions: Indol negative. Do not assimilate peptone in absence of carbohydrates. Catalase negative. Nitrates not reduced to nitrites. Hydrogen sulphide formed from sulphites, thiosulphates and oatmeal. Acetyl-methyl-carbinol not present. Gelatin not liquefied. Ammonium salts not utilized.

Dissimilation of carbohydrates: Acid and gas from glucose, lactose, maltose, sucrose, xylose, levulose, galactose, melibiose, trehalose, amygdalin, dextrin, starch, esculin, inositol, inulin, salicin, glycogen, dimethylglucoside, cellobiose, arabinose, raffinose, melezitose, rhamnose.

No acid or gas from pectin, mannitol, adonitol, dulcitol, erythritol, sorbitol, glycerol or sodium lactate.

Final products: Butyl alcohol, isopropyl alcohol, carbon dioxide, hydrogen, small amounts of butyric and acetic acids; may be traces of formic acid and acetone.

TABLE 1. *Fermentation of glucose in 2 per cent solution; 0.7 per cent peptone; 0.2 per cent yeast extract; 0.1 per cent di-potassium phosphate. Products expressed in millimoles per 100 millimoles of glucose fermented*

Glucose fermented	Products per 100 mM. of glucose							Carbon recovered	Redox index*
	Butyl alcohol	Isopropyl alcohol	Ethyl alcohol	Butyric acid	Acetic acid	CO_2	H_2		
mM.	mM.	mM.	mM.	mM.	mM.	mM.	mM.	pctg'	
100	57.6	15.1	2.9	15.1	9.1	166.5	75.8	87.0	.86
100	66.6	15.1	0	6.1	21.2	197.0	72.7	95.0	1.02
100	58.6	12.1	0	17.2	17.2	203.5	77.6	96.3	1.06
100	54.6	15.1	2.9	18.2	12.1	200.0	121.2	93.5	.93
100	65.5	13.8	2.3	13.8	10.3	189.5	86.2	95.6	.90

* cf. Erb, Wood and Werkman, J. Bact. 31, 595 (1936).

DISSIMILATION OF GLUCOSE

Data for the fermentation of glucose in 2 per cent solution (0.7 per cent peptone, 0.2 per cent yeast extract and 0.1 per cent K_2HPO_4) are given in table 1. The results are given in millimoles of products formed per 100 millimoles of glucose fermented for comparative purposes. These experiments were not carried out at the same time and unintentional changes in environmental conditions probably account for the varying yields of products. Addition of intermediary substances in large quantities to fermenting glucose imposes a significant change in environmental conditions, and in such experiments wide variations in yields of products are to be expected. High yields of acids are obtained obviously at the expense of the alcohol and it is necessary to consider both the alcohols and acids produced in interpreting the experiments given below.

Fermentation of acetic and butyric acids. Low concentrations of free unbuffered acid are toxic to *Cl. butylicum.* Addition of the sodium or calcium salts of the acids led to recovery of acid salt equivalent, though not necessarily identical, to the salt added. In solutions buffered with di-potassium phosphate, free acids can be present in significant quantities without inhibiting growth. In figure 1, curve IV was obtanied by titrating 20 cc. of 1 M di-potassium phosphate with 1 N hydrochloric acid. Curves I

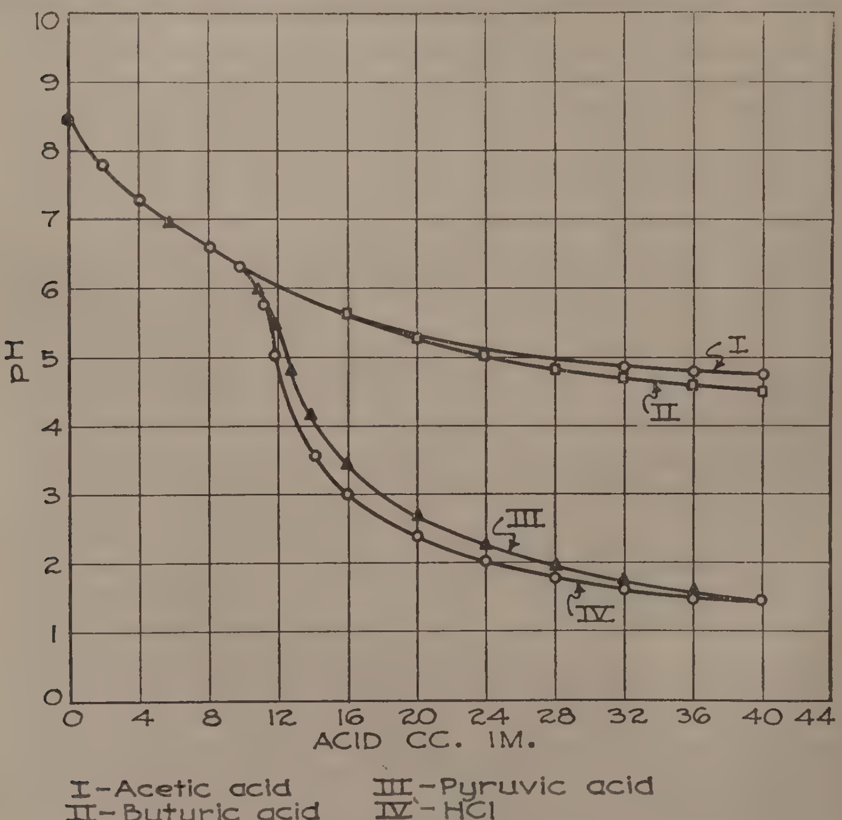

Fig. 1. Titration curves of acids

TABLE 2. *Fermentation of acetic and butyric acids in presence of glucose*

No.	Glucose fermented	Acid added per 100 mM. of glucose		Production in mM. per 100 mM. of glucose						K_2PO_4 added cc. 1 M	pH		Carbon recovered	Redox index
		Acetic	Butyric	Butyl alcohol	Isopropyl alcohol	Butyric acid	Acetic acid	Carbon dioxide	Hydrogen		Initial	Final		
	mM.	mM.	mM.										pctg.	
1	100	0	0	50.2	18.0	14.5	20.3	207.0	111.0	0	6.9	4.3	90.4	1.05
2	100	0	0	54.1	18.0	15.5	12.9	222.0	107.0	0	6.9	4.3	96.2	1.08
3	100	0	38.5	71.2	25.7	7.7	30.9	223.5	82.2	33.4	5.4	5.5	90.0	1.14
4	100	0	64.3	86.8	29.6	9.6	38.6	241.0	....	55.0	5.1	5.4	93.0	....
5	100	0	41.5	70.6	22.7	22.2	38.7	227.0	104.2	55.5	6.3	5.4	97.5	1.36
6	100	43.2	0	47.2	48.0	12.5	12.0	233.0	74.5	36.0	5.5	6.3	93.4	1.30
7	100	65.3	0	54.1	47.8	12.0	20.3	214.0	....	45.0	5.0	5.7	90.6	....
8	100	65.3	0	56.4	49.6	6.8	11.7	219.0	....	45.0	5.0	6.4	88.3	....

TABLE 3. *Fermentation of pyruvic acid by Cl. butylicum*

	1	2	3
Pyruvic acid added mM.	27.0	40.0	34.0
Pyruvic acid fermented mM.	26.0	40.0	32.0
Acetic acid mM.	10.5	20.4	20.0
Butyric acid mM.	7.0	7.5	6.0
Carbon dioxide mM.	25.7	41.5	32.5
Hydrogen mM.	5.4	11.7	9.5
Carbon recovered percentage	94.5	93.5	100.5

and II were obtained by titrating 20 cc. of the phosphate with 1 N acetic and butyric acids, respectively. With a given quantity of phosphate present, acetic or butyric acid in excess of that required to bring the mixture to pH 6.3 will exist in solution as free acid, and as such will be subject to fermentation.

Experiments in which acetic and butyric acids were added to fermenting glucose in solutions buffered with di-potassium phosphate are shown in table 2. Results of fermentations were calculated to a common basis of compounds added and products formed per 100 millimoles of glucose fermented. Experiments 1 and 2 are controls. The addition of butyric acid in experiments 3 and 5 gave an increase in the yield of both butyl and isopropyl alcohols. An increased quantity of acetic acid was also formed in each flask. The greater yields of isopropyl alcohol and of acetic acid indicate a shifting of the reactions in such manner that more than the normal quantities of 2-carbon compounds were produced from glucose, and not the conversion of butyric acid into acetic acid. No biochemical reactions are known for the latter conversion. Experiments 6 to 8 show an increase of approximately 30 millimoles of isopropyl alcohol in each experiment. Since two molecules of acetic acid are required to produce one molecule of isopropyl alcohol it is evident that complete conversion of acetic acid to alcohol has occurred.

Fermentation of pyruvic acid. Table 3 shows results for the fermentation of pyruvic acid.

In experiment 1, 27 millimoles of calcium pyruvate in 200 cc. of solution were fermented by a cell suspension of *Cl. butylicum.* The organisms had been grown in 12 liters of 2 per cent peptone broth for 24 hours and separated by the supercentrifuge. No nitrogen source was added to the pyruvate solution.

The formation of hydrogen equivalent to half of the acetic acid indicates that a salt of formic acid was formed which, under the conditions of these experiments, gave one-half molecule H_2 for each formate molecule formed. If pyruvic acid had been decarboxylated to form acetaldehyde, and aldehyde had been converted into acetic acid, the reactions would be:

$$CH_3COCOOH \longrightarrow CH_3CHO + CO_2$$

$$CH_3CHO + H_2O \longrightarrow CH_3CH(OH)_2 \longrightarrow CH_3COOH + H_2$$

$$2CH_3CHO \longrightarrow CH_3CHOHCH_2CHO \longrightarrow CH_3CH_2CH_2COOH$$

Under these conditions the millimoles of hydrogen formed should equal the millimoles of acetic acid, and there would be 10.5 H_2 in experiment 1 of table 3. The sodium salt of pyruvic acid was used in experiment 2, and in experiment 3, a calcium pyruvate medium in the presence of peptone was inoculated and fermented in the same manner as glucose solutions. In all of these experiments the hydrogen was equal to approximately one-half of the acetic acid formed. All of these experiments show that pyruvic acid was converted into mixtures of acetic and butyric acids. Since the acids all existed as salts, there was no further conversion into alcohols.

Fermentation of pyruvic acid in presence of fermenting glucose. Experiments in table 4 show that pyruvic acid was converted into 2- and 4-carbon compounds in about equal proportions. Calcium pyruvate was added to experiments 2 and 3, and as was to be expected, relatively large yields of volatile acids were obtained. Under the headings A and B, the calculated millimoles of 4- and 2-carbon compounds are given and it is evident that, with one exception, the ratios of 4- to 2-carbon compounds were not greatly altered by the addition of pyruvic acid. To experiments 4 and 5 pyruvic acid and di-potassium phosphate (see footnotes 2 and 3 to table 4) were added. Curve III of figure 1 shows that pyruvic acid cannot be buffered with di-potassium phosphate. If, as is shown by table 4, the pyruvic acid salt is converted into mixtures of acetic and butyric acids, these two acids will be buffered slightly by the amounts of phosphate present. More alcohols and less volatile acids should be formed in experiments 4 and 5 than in 2 and 3, but less alcohol and more acid in both cases than in experiment 1 of table 4, which was found to be true. The experiments in tables 3 and 4 show conclusively that pyruvic acid is converted into both 2- and 4-carbon compounds.

Fermentation of acetaldehyde in presence of fermenting glucose. If

TABLE 4. *Fermentation of pyruvic acid in presence of glucose. Products expressed in millimoles*

	1 Control	2 15 mM.[1] Pyruvic acid	3 20 mM.[1] Pyruvic acid	4 15 mM.[2] Pyruvic acid	5 20 mM.[3] Pyruvic acid
Glucose fermented mM.	36	36.4	38.3	38.3	35.0
Butyl alcohol	22.6	16.4	16.1	20.8	20.0
Isopropyl alcohol	8.5	5.0	4.9	8.2	7.0
Acetic acid	4.0	10.2	9.0	8.0	10.0
Butyric acid	3.0	13.0	19.0	8.0	6.0
B	25.6	29.1	35.1	28.8	26.0
A	21.0	20.2	18.8	24.4	24.0
Carbon dioxide	72.0	92.0	94.0	91.0	100.0
Hydrogen	36.0	37.0	40.1	33.5	37.0
Final pH	4.9	4.4	4.9	6.0	5.5
Carbon recovered percentage	95.4	93.0	92.0	92.0	90.8

[1] Calcium pyruvate added.

[2] 15 cc. 1 M pyruvic acid +16.8 cc. 1 M K_2PO_4.

[3] 20 cc. 1 M pyruvic acid +22.6 cc. 1 M K_2PO_4.

TABLE 5. *Fermentation of acetaldehyde in presence of glucose. Substrates and products in millimoles*

	Control 1	2	3	4
Glucose fermented	32.8	36.6	35.6	26.4
Acetaldehyde fermented*	0.0	10.0	7.0	8.75
Butyl alcohol	18.5	15.0	17.8	15.0
Ethyl alcohol	0.8	7.5	6.5	7.0
Isopropyl alcohol	5.5	10.5	2.0	3.3
Acetic acid	3.3	4.3	5.0	3.0
Butyric acid	3.3	2.3	4.5	5.7
Acetylmethylcarbinol	absent	0.5	trace	trace
2, 3-Butyleneglycol	absent	1.0	absent	absent
Carbon dioxide	74.0	80.0	65.0	62.0
Hydrogen	34.8	30.0	27.0	26.6

* 10 mM. aldehyde added to each of last three experiments.

the reactions of fermentation are as postulated by former workers, it seems that acetaldehyde should give mixtures of isopropyl and butyl alcohols when added to glucose fermentations. Table 5 shows that acetaldehyde was converted almost quantitatively into ethyl alcohol. Small quantities of acetylmethylcarbinol and of 2, 3-butylenegylcol were also formed.

Fermentation of acetone in presence of fermenting glucose. The addition of 15.6 millimoles of acetone to experiments 2 and 3 in table 6 shows its conversion into isopropyl alcohol. There was no corresponding decrease in molecular hydrogen. The smaller yields of butyl alcohol may account for the hydrogen consumed in converting the acetone into isopropyl alcohol.

Acetoacetic acid was prepared from the ethyl ester by hydrolysis with potash. In one experiment the acetoacetic acid was converted into isopropyl alcohol when added to fermenting glucose solution.

Lactic acid was not fermented by *Cl. butylicum* under any conditions tried. When it was added to fermenting glucose an equivalent quantity of lactic acid was always recovered.

DISCUSSION

It is to be understood that interpretation of the data in this paper is affected by the possibility that addition of reagents to fermenting glucose solutions may have altered the normal course of the fermentation. The isolation of methylglyoxal and the accumulation of lactic acid in large quantities in the presence of sodium bicarbonate indicate (1937) the existence of methylglyoxal as an intermediary compound under the conditions imposed. Failure of the organisms to ferment lactic acid must mean that the lactic acid arose from the methylglyoxal because of the environmental conditions imposed by the bicarbonate, and that normally the reactions do not proceed through a lactic acid stage. Formation of acetic and butyric acids or of butyl and isopropyl alcohols from pyruvic acid does not necessarily mean that pyruvic acid is an intermediary product in the formation of these substances from glucose: that pyruvic acid

is not excluded as an intermediate, however, deserves consideration. In contrast, some attempts failed completely when effort was made to isolate acetaldehyde from fermenting glucose and the quantitative formation of products from acetaldehyde which are not produced in significant quantities from glucose. If acetaldehyde had played an important intermediary role in the formation of 4-carbon compounds there would have been an increase in such compounds shown in table 5.

Decarboxylation of pyruvic acid to form acetaldehyde, with subsequent conversion of the aldehyde to ethyl alcohol, can account for the small quantities of ethyl alcohol produced in butyl-isopropyl acohol fermentations.

Reactions indicated in the butyl-isopropyl alcohol fermentation are:

$$C_6H_{12}O_6 \longrightarrow 2CH_3COCHO + 2H_2O$$

$$CH_3COCHO + H_2O \longrightarrow CH_3COCOOH + 2H \text{ (or } H_2\text{)}$$

$$CH_3COCOOH + H_2O \longrightarrow CH_3COOH + HCOOH$$

$$HCOOH \longrightarrow H_2 + CO_2$$

$$2CH_3COCOOH \longrightarrow CH_3CH_2CH_2COOH + 2CO_2$$

$$2CH_3COOH \longrightarrow CH_3COCH_2COOH \longrightarrow CH_3COCH_3 + CO_2$$

$$CH_3COCH_3 + 2H \longrightarrow CH_3CHOHCH_3$$

$$CH_3CH_2CH_2COOH + 4H \longrightarrow CH_3CH_2CH_2CH_2OH + H_2O$$

$$CH_3COCOOH \longrightarrow CH_3CHO + CO_2$$

$$CH_3CHO + 2H \longrightarrow CH_3CH_2OH$$

Formation of butyric acid from two molecules of pyruvic acid through the intermediary stage of pyruvic acid aldol has been discussed by Neuberg and Arinstein (1921).

SUMMARY

Normal end products of the butyl-isopropyl alcohol fermentation are butyl and isopropyl alcohols, carbon dioxide, hydrogen, and small amounts of butyric and acetic acids and ethyl alcohol.

TABLE 6. *Fermentation of acetone in presence of glucose. Substrates and products in millimoles*

	Control 1	2	3
Glucose fermented	38.8	36.2	37.0
Acetone added	0	15.6	15.6
Acetone fermented	....	13.9	12.0
Butyl alcohol	24.6	18.4	20.0
Isopropyl alcohol	7.6	22.5	21.5
Acetic acid	3.5	5.0	7.0
Butyric acid	4.0	4.0	7.5
Carbon dioxide	84.4	82.5	83.7
Hydrogen	32.2	29.3	30.5

Butyric acid added to fermenting glucose is reduced to butyl alcohol. Presence of butyric acid in fermenting glucose solutions has a pronounced effect on the course of the fermentation reactions, leading to high yields of isopropyl alcohol from glucose.

Acetic acid is converted into isopropyl alcohol. Addition of acetic acid did not affect the yields of butyl alcohol.

Acetaldehyde was converted into ethyl alcohol, with traces of acetylmethylcarbinol and 2, 3-butyleneglycol.

Acetone was converted into isopropyl alcohol. Lactic acid was not fermented under any conditions.

Pyruvic acid was converted to butyl and isopropyl alcohols when added to fermenting glucose. When fermented alone the sodium or calcium salt of pyruvic acid yielded a mixture of acetic and butyric acids.

Chemical reactions suggested by the data obtained are given.

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THE FOOD AND COVER RELATIONSHIP IN THE WINTER SURVIVAL OF THE RING-NECKED PHEASANT, PHASIANUS COLCHICUS TORQUATUS GMELIN, IN NORTHERN IOWA[1]

WILLIAM EDWARD GREEN[2]

From the Entomology and Economic Zoology Section, Iowa Agricultural Experiment Station

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With the passing of the abundance of the Greater Prairie Chicken, *Tympanuchus cupido americanus* (Reichenbach), in northern Iowa, exotic species of game birds became more attractive to farmers and sportsmen alike. The success of the Ring-necked Pheasant, *Phasianus colchicus torquatus* Gmelin, was so marked and its game qualities were so satisfactory that assistance with the management and maintenance of shootable numbers of the pheasant developed into one of the major activities of the Iowa State Conservation Commission. Because of the realization of the difficulties of this activity and in appreciation of the results obtained in previous cooperative agreements between Iowa State College, the former Fish and Game Commission, and Mr. J. N. Darling, the cooperative program of research and education was enlarged in 1935. The American Wildlife Institute, cooperating with the United States Bureau of Biological Survey, the Iowa State Conservation Commission, and Iowa State College agreed to participate equally in a general wildlife management program.[3] Within the major activity concerned with the Ring-necked Pheasant, a project to work out a pheasant management plan that would be practical and satisfactory to both the farmers and sportsmen was established as a part of the cooperative program. To conduct research in the development of such a plan it was decided to employ a graduate assistant in the Entomology and Economic Zoology Section of the Agricultural Experiment Station, Iowa State College, and the author was called to this position on October 21, 1935.

The research was pursued under the guidance of Dr. George O. Hendrickson, Assistant Professor in Wildlife Management, Iowa State College, and Dr. Logan J. Bennett, Associate Biologist, United States Bureau of Biological Survey. The writer is greatly indebted to Dr. Carl J. Drake, Head of the Department of Zoology and Entomology, Iowa State College, for invaluable help in the accomplishment of this work, and to Assistant Game Supervisor, F. H. Davis, of Clear Lake, Iowa, for suggestions made during the study. In addition, the author wishes to express his thanks to the farmers on the area, especially the key men, Harold

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Hove and O. B. Christenson, whose cooperation and support made this study possible.

As an area on which to carry out the research in the establishment of a working pheasant management plan, there was chosen for the author a tract of 4900 acres, sections 9, 10, 14, 15, 16, 21, 22, and 23, Range 25, Township 100, Eden Township, and seven miles north of Thompson, Winnebago County. A plot of the area with the arrangement of farms and names of the farmers was prepared and reproduced in Plate I.

The most nearly accurate information concerning the introduction of the pheasant into the area was obtained from Lars Flo, a farmer living in section 15. Mr. Flo stated that in 1909 or 1910 pheasants were released at Coon Grove, a native stand of timber surrounded by much poorly drained untilled marsh land, located one mile east and four miles south of the chosen tract. From this planting, he presumed, the birds spread into the area included in this investigation.

Before recommendations in regard to management could be given it was deemed advisable to follow the pheasants through the winter in a survey of the factors which affected the welfare and the numbers of the birds. It became quite apparent to the author, after holding conversations with several of the farmers, that they did not carry out any practices specifically intended to benefit the pheasants; i. e., the pheasants cared for themselves. The same conditions were found by the Iowa Fish and Game Commission (1932), which states that, "The average Iowa farm automatically provides some food and cover for upland game, but unless proper cover and food are present at critical seasons, game will not survive." Hence the author decided to watch particularly the food and cover relationship in the winter survival of the Ring-necked Pheasants of the area.

The winter was the most severe recorded in the history of the state (Reed, 1936). In the late fall and through the first half of January, 1936, the weather was nearly normal. On January 18 a cold period began in which the temperatures dropped well below zero. During this cold wave which continued almost unabated through February 22, Iowa experienced the most protracted period of very low daily temperatures that have been recorded for the state. February was the coldest of the 117 Februaries on record. In addition strong cold winds and heavy snowfall continued throughout most of the month. A blizzard on February 8, extending over the major portion of the state, and a second on February 26, striking most severely in the north-central and north-west counties, were described by inhabitants as the worst in 50, or more years.

TECHNIQUES OF INVESTIGATION

The author was in the field every day from November 11, 1935, to March 5, 1936, except for a few clear Sundays and two very stormy week days. Observations were made from about 7:30 a.m. until 5:30 or 6:00 p.m., as a rule, and these were supplemented by a few observations before daylight and after dark.

The Harold Hove farmstead, at which the author resided, was located at the southeast corner of section 16 and fairly near the center of the area. This location facilitated work because in a forenoon or afternoon a complete circle could be made on the sections to the east or to the west. In

order to gather more varied and more nearly accurate observations, the path traversed was changed from day to day.

Daily notes were taken on the following points: methods used by the investigator; the numbers and locations of pheasants observed, together with their physical condition and activities; and prevailing weather conditions.

During late fall, counts of the pheasants were made on foot or on horseback, with the aid of a dog. The few fields that could be entered most conveniently were covered quite well by riding back and forth several times. This technique worked the birds to one end of the field where they could be counted as they flushed. Because most of the farms were well-fenced, it was inconvenient to take a horse from one farm to another across the fields, and hence it was more economical of time, after coming near the center of a section on horseback, to go over the remaining farms of the section on foot.

With the advent of colder weather and heavy drifts of snow it became impossible to use a horse at all. By January 19, 1936 the snow was drifted so deeply in places that it was difficult to even get around on foot. Accordingly, skis were worn on the trips, and with them, the author traveled an average of 15 miles a day.

The farm dog that was used, although not well-trained, proved valuable in making pheasant counts. On trips, it ranged some distance from the investigator, and consequently birds were flushed from much more ground in a given time. In later winter the dog was seldom taken along because weather conditions were often such that it was not advisable to flush the birds.

In the fields the following method of counting was used in late winter with good success. A field, usually of corn, was generally entered at one corner, and then a zig-zag course through the field was taken until the opposite end was reached. The pheasants were counted as they flushed from the end of the field.

In addition to the counts in the fields, daily counts were made of the pheasants at various concentration centers mornings or evenings during the period from late November to the last of February.

COVER OF THE AREA

Cover as associated with game has attained a variety of meanings and limitations. The author has been guided by the dictionary definition of cover, which is in accord with Leopold (1933), who defined cover to mean vegetative or other shelter for game. In order to determine the parts of the environment used as cover by the pheasants, it appeared advisable in the beginning to consider all parts as possible cover.

A wide variety of cover was found on the area. Perhaps the 1480 acres of plowed fields and other barren ground such as harvested beet and tomato fields served as cover at times, but pheasants were observed sitting in plowed fields only once. On November 20, 1935, the author, with Logan J. Bennett, traversed a four-acre field of unmown sweet clover. Four birds flushed from there and flew to a plowed field where they remained for some time. A 10-inch snowfall on November 27 covered these plowed and barren fields, and from then until about March 15, 1936, the fields remained mostly covered with snow. During that time,

although birds were observed walking across such fields, they were not observed to tarry there.

The stubble cover included 364 acres of oat stubble, 140 acres of weedy corn stubble, 7 acres of flax stubble, 109 acres of sweet clover 15 inches tall, and 171 acres of mown alfalfa. Before the snowfall of November 27, 1935, birds often were observed to linger in these fields, but after that the fields were covered with snow and no birds were seen loitering there.

On the area were 292 acres of pasture land. These pastures were closely grazed and cover there was slight. Before cold weather, pheasants were sometimes flushed from the pastures, but after the snow of November 27, the pastures were filled with snow and birds could no longer be flushed from them.

Hayfields other than alfalfa comprised 121 acres of the area. These fields were mown and so closely grazed that the remaining stubble was very short. Pheasants were flushed from these hayfields before the snow of November 27, but after that no birds were put up at such places.

In the fall before snow came, pheasants were frequently seen, especially in cold or windy weather, at fence rows and the banks of shallow ditches along the roads. Birds were flushed from such places mostly during the day. Sometimes in early morning pheasants were observed on the bare, over-hanging banks of roadside ditches and along the east side of fence rows in the fields. Most of the fence rows and roadside ditches were grown to weeds and tall grasses. However, such places tended to drift over in late fall and following that were not used as cover. By November 26, fences and ditches running north and south were filled with snow, so that they were worthless for cover. Those running east and west also filled rapidly and by December 12, birds were no longer flushed from such places.

On the Harold Hove farm, section 16, was a four-acre patch of dense unharvested sweet clover (*Melilotus alba*) over six feet tall (Plate III, figure A). The tract was about five rods wide and about 120 rods long. Before drifting of snow occurred, an average of 20 birds were flushed from this patch daily. The time of day appeared to make little difference in the number of pheasants which could be flushed from this tract, although the highest numbers were flushed at mid-day and just before dark. Pheasants were not seen there after the patch drifted full of snow (Plate III, figure B).

Of several sloughs, only three unmown, lightly grazed tracts harbored pheasants. The slough on the Leonard Olson farm, section 16, sheltered many birds early in the winter. Counts made in early morning and evening indicated an average of 60 pheasants flushed from this place before the hunting season and an average of 30 were put up daily for several weeks after the season. The vegetation of this slough consisted chiefly of bulrushes (Scirpus spp.) bordered by sedges (Carex spp.) and slough grass (*Spartina Michauxiana*). As early as December 12, the slough was full of snow and pheasants were no longer found there.

The other two sloughs lay close together on the Chris Walle farm, section 15. The vegetation of these sloughs consisted chiefly of tall reeds *(Phragmites communis)* bordered by unmown, lightly grazed slough grass. These sloughs remained open longer than the first mentioned

slough because of excellent protection offered by a nearby willow row at the west. Before the hunting season about 50 birds spent the nights in these two sloughs and at the willow row, and after that about 35 were sheltered there nightly for two months. In windy or cold weather some birds could be flushed from these sloughs during the daylight hours. After February 8, 1936, the sloughs, drifted full of snow, were no longer used by pheasants.

Around the edges of the sloughs the short slough grass was about eight inches tall. Before this short grass drifted full of snow the birds were nearly always flushed from it and seldom from the tall reeds. Droppings indicated that the birds stayed in this short grass at night. During early morning and late evening trips, pheasants were quite frequently put up from this grass. After the short grass filled in with snow the tall reeds (Plate III, figure C) harbored pheasants during the night until January 21. At that date, heavy drifting filled in the stand of reeds and from then until late winter both sloughs were full of snow and pheasants were not found in them.

The 1450 acres of harvested corn had some cover value. About three-fourths of the corn was hand-picked and the remainder was machine-picked. Except for one 40-acre field of hand-picked sweet corn, the corn was of field varieties. In the machine-picked corn the stalks, badly crushed and broken down were covered almost completely with the first heavy snow, January 19. Hand-picked corn, on the other hand, offered more cover than the machine-picked corn throughout the winter. Snow in the hand-picked fields by February 10, averaged three to four feet in depth, but two to four feet of cornstalks remained above the snow. Cattle grazed in nearly all the cornfields until about December 1, 1935. The contrast between hand-picked sweet corn and machine-picked field corn is shown in Plate III, figure D. The machine-picked field was almost entirely drifted over whereas stalks in the hand-picked field were quite prominently in view. The sweet corn field sheltered as high as 175 pheasants daily early in the winter, but only about 96 were flushed each day from the field later.

In mild weather pheasants were not found at strawstacks. With the coming of severe cold, however, many pheasants went to the stacks. Especially was this true in cold windy weather when the birds huddled on the leeward side during strong winds. It was common for pheasants to burrow not more than half-way into the straw, usually near the top of the stack (Plate IV, figure C). Pheasants were found in such places both at night and during the day.

Close to farm buildings were situated several groves of evergreens of varying size and density. These groves contained the following trees, usually in more or less mixed stands: Scotch pine (*Pinus sylvestris*), white spruce (*Picea glauca*), Norway spruce (*Picea abies*), Colorado blue spruce (*Picea pungens*), White fir (*Abies concolor*), and Douglas fir (*Pseudotsuga taxifolia*). The author did not observe any use of evergreen groves by pheasants for any kind of shelter during the winter. Even in the most severe weather the birds used other shelter although such shelter was farther from the feeding areas than were the evergreens. None of the evergreen groves abutted a field which contained food available to the birds. Two farmers reported that during the winter of 1934-'35, pheasants had used evergreen groves that were contiguous with corn-

fields. In England, Tegetmeier (1904) reported in detail on the successful use of evergreens for pheasant roosting cover. He recommended spruces and firs highly and assigned some value to close branching pines, but made no mention of the proximity of food to the evergreen cover.

Although every farmstead on the area had a grove of deciduous trees, only two such groves near occupied buildings showed evidence of use as cover by pheasants. The large grove of the Lars Flo farm, section 15, sheltered nine pheasants regularly night and day during very cold weather. With the advent of warm weather the birds were no longer found there. On the Carlson farm, section 9, a mixed coniferous and deciduous grove was situated west of the buildings. On November 13, the author observed 34 pheasants perching in a willow row on the extreme western edge of the grove, but at no other times were pheasants seen in that grove. At the unoccupied farmstead of the 40-acre tract, section 22, leased by Morris Erdol, eight pheasants were found on three occasions at 8:00 a.m. These birds were huddled on the southeast corner of the grove during strong winds.

On the Levi Selvig farm, section 9, a three-acre deciduous grove, without buildings, sheltered about 75 pheasants throughout the winter. Birds were usually seen there before and during storms, but numerous droppings indicated that they were also spending the nights there. Tall cottonwoods *(Populus deltoides)* and tall elms (Ulmus spp.) were the major trees of this grove. Rose bushes (Rosa spp.) and tall burdock (*Arctium minus*) added to the cover in the grove. (Plate IV, figures A and B.)

Willows (*Salix amygdaloides*) were used throughout the winter. A willow row (Plate IV, figure D) located just south of the Chris Walle home, section 15, occasionally harbored 20 pheasants during storms. These pheasants were from the flock that fed regularly in the 60-acre handpicked cornfield across the road. A shorter row of second growth willows (Plate V, figure A) situated just across the road from the willow row of Plate IV, figure D and at the edge of that cornfield, was not used by the birds. At no time did the author observe pheasants in this willow row, regardless of weather conditions or time of day. But the single willow tree and all its shoots (Plate V, figure B) located in almost the exact center of the afore-mentioned 60-acre cornfield provided cover for 14 to 34 pheasants throughout the winter, even in severe weather. Birds were flushed from there in the daytime, and because they were found there in early morning and late evening, it is probable that they spent the nights near that tree. A clump of first growth willows covering an area of nearly two square rods and located on the Olson Bros. farm, section 21, was much used by pheasants at night and during the daytime in the earlier part of the winter. Until it drifted over, that clump harbored about 105 birds at night and during stormy days. The birds utilized the clump as cover until it was completely drifted over. In Plate V, figure D, and Plate VI, figures A to D, inclusive, that willow clump is shown in the successive stages of drifting. On the Elveback Bros. farm, section 22, a willow row located 10 rods east of the buildings sheltered seven pheasants during storms and at night (Plate V, figure C). At the western fence of the Chris Walle farm, section 15, a willow row (Plate VII, figure A) located on the west side of the sloughs was used by pheasants regularly at night

and somewhat during the day, especially in stormy weather. It was also of value because it prevented the sloughs from drifting full until the severe drifting that occurred February 8, 1936. The willows, partly filled with snow earlier in the winter, were nearly covered with snow on February 8, but they continued to give protection to the sloughs.

CLASSES OF COVER

With respect to function, Leopold (1933) defined several classes of cover of which the following were found in this investigation: (1) escape cover, that offered invisibility, mechanical protection from precipitation, and refuge from hunters; (2) loafing cover, that offered wind protection and sun in winter; (3) roosting cover, that offered a resting place at night.

The pheasants apparently had little need for escape cover in which to hide from preying wild animals. The only raptorial birds seen on the area were two American Rough-legged Hawks, *Buteo lagopus s. johannis* (Gmelin), and five Short-eared Owls, *Asio flammeus flammeus* (Pont.). The hawks ranged over the southeastern section and neighboring land outside the area, while the owls were found in the grove of the Fred Lamping farm, section 21. No other predatory wild animals were observed during the winter. At no time were live pheasants noticed to be disturbed by a predator. Hence it was not possible to rank the escape value of the cover for pheasants in relation to actual use for invisibility or mechanical obstruction to ward off preying enemies. For mechanical protection against snow the behavior of the birds gave more evidence. Generally several hours before a snowfall pheasants began to congregate at willow rows and clumps, in sloughs, in groves, and in the unmown sweet clover. So constant were those movements that farmers predicted coming snows by observing concentrating pheasants at those places. The only other cover in which pheasants remained during snowfalls was the sweet corn field which the feeding birds did not leave before the storms. As no fields were set off by the farmers as refuges, the hunters had free access to all parts of the area. No cover of itself offered much refuge to the birds from the hunters, for during the hunting season the gunners were able to drive birds out of all cover types.

Several types of cover served for loafing purposes. During periods of strong, cold winds, pheasants sought shelter at the leeward sides of groves, willows, and straw stacks, and in the sloughs. The birds left such shelter to feed for only a few hours on windy days. Pheasants were seen to tarry at times, particularly in mornings, at the sunny sides of ditch banks, weeds of fence rows, and other taller vegetation.

The pheasants generally roosted on the ground. When the weather was mild, as in late fall, slightly more than one-half of the birds rested on the ground at nights in cornfields, stubble fields, and short slough grass. The remainder of the pheasants in such weather roosted on the ground at the willows and groves, and in unmown sweet clover. After the onset of cold winter weather many of the birds roosted on the ground or snow in the shelter of the willows, groves, sloughs, and unmown sweet clover. A varying number of pheasants roosted in the unpicked sweet corn field at night, in even the coldest weather. A few pheasants burrowed into straw-stacks to rest during several very cold windy nights.

On only one occasion, at 10:30 a. m. on a misty late fall morning, were pheasants seen in trees. Perhaps they roosted there the previous night.

Leffingwell (1928) observed that in Oregon the pheasants were roosting in trees to quite an extent, although ground roosting appeared to be more common.

FOOD

The food of the pheasants was quite varied during the winter months. Much food was available on every farm in early fall, although some places offered a greater abundance than others.

In the three-acre vacant grove, section 9, were dense stands of burdock *(Arctium minus)* as shown in Plate VII, fig. D, of which the seeds were probably used as food for pheasants. To secure the seeds, perhaps the pheasants rolled the burs around until they matted together (Plate VII, fig. B), for many pheasant tracks were seen around these mats. No crops of pheasants found dead at this grove were examined to supply further information concerning the food of the birds at that location.

Smartweed (Polygonum spp.) was of some importance as winter food, especially in sheltered spots. It was common in some stubble fields, and was available early in the winter. However, because of its short growth it soon was drifted over and the seeds were then unavailable to the pheasants.

On section 22 the crop of a four-acre patch of soybeans (*Soja max*), was piled in many cocks which were at least partly above the snow throughout the winter. During the first part of the winter the cocks were not visited by pheasants, but as other food became scarcer there was considerable evidence of pheasants feeding on the beans. Pheasant tracks were numerous around the piles of beans and broken pods were scattered on the ground. No crops of birds from this field were examined.

There was some evidence that pigweed *(Amaranthus retroflexus)* was used as food, especially on the banks of the ditch on Olson Bros. farm, section 21. From the tracks and bits of plant material scattered on the ground it appeared on several days that pheasants ate some of the pigweed seeds.

Seeds of lamb's quarters *(Chenopodium album)* served as food throughout the winter, even where corn was available. This weed was prevalent in cornfields, in thin willow thickets, on banks of and in shallow ditches, and in fence rows. Crops that were examined showed this seed to be present.

In small grain fields the lesser ragweed *(Ambrosia artemisiifolia)* was quite common. A small stand of greater ragweed *(Ambrosia trifida)* was found on the ditch banks, Olson Bros. farm, section 21. Crops that were examined showed seeds of both ragweeds as present, with the lesser ragweed far more abundant than the greater one.

Pigeon grass (Setaria spp.) was taken as food in considerable quantities. Its presence in corn rows in all cornfields on the area no doubt had a great influence on its popularity. Crops that were examined contained an abundance of pigeon grass seed.

Oats *(Avena sativa)* ranked next to corn in the pheasant's diet. Examined crops had a large proportion of oats. These oats were picked up, quite surely, at or near strawstacks, and some stacks yielded more grain than others.

On most of the farms of the area, the hogs were disposed of early in the season or else kept out of cattle yards during the severe weather. Hence, the manure in the cattle yards and barns was not cleaned of corn. When this was hauled to the fields considerable corn was made available to wildlife. It was common to see numbers of pheasants feeding on freshly hauled manure. Because of the extremely cold weather the manure soon froze and the corn could not be taken out by the birds and was therefore of food value only for a short time after the spreading.

Corn was, beyond a doubt, the basis of subsistence of the pheasants under observation. Both field and sweet corn were utilized by the birds. When corn was not available in the fields, the birds entered hog lots, visited corn cribs, and frequented manure piles in an effort to obtain this cereal. On the area there seemed to be no shortage of corn as food. Machine-picked corn offered considerable food in early fall and winter because so much was shelled off during the picking process. The snow of November 27 partly filled these fields and by December 12, they were so full of snow that the corn was unavailable to the birds. In the early winter hand-picked corn did not offer as much food as did the machine-picked corn. Because the food of the hand-picked fields consisted of unpicked ears hanging above the snow, the actual available supply throughout the winter was much higher. A general view of a hand-picked field is shown in Plate VIII, fig. B. When this picture was taken the fields were so full of snow that most of the ears were either below or just above the top of the snow. While the available food is not all apparent in this picture, this field offered a large amount of food to the pheasants. In one hand-picked field of 60 acres, an average of nearly one ear to the hill was left in late and hurried picking (Plate VIII, fig. A). In the corn rows there was also much pigeon grass and lamb's quarters of which the seeds were used as food. The 40-acre field of sweet corn, section 22, contained much unpicked corn left after selective picking. In this sweet corn many entire hills were left unhusked. Such views as Plate VII, fig. C, which show kernels missing from the ears and many pheasant tracks around the hill, were common throughout the field. During the first part of the winter the pheasants were very particular about the quality of the corn eaten in this field. With plentiful food before them, only the choice kernels were taken. Frequently, ears were seen with only the good kernels taken and with all smutty, moldy, cracked, or otherwise damaged kernels left. Later, when food became scarcer, the pheasants visited these cobs again and picked out even the poor kernels.

Listed in the order of importance, seeds of the following plants were taken by the pheasants for food: field corn, sweet corn, oats, pigeon grass, corn (in manure), lesser ragweed, lamb's quarters, smartweed, soy beans, pigweed, and greater ragweed.

Similar observations have been made in other states. Leffingwell (1928) reported that pheasants observed in several states seemed to feed on whatever was easiest to obtain, but that the three seeds most commonly eaten were ragweed, smartweed, and foxtail. Severin (1933) found that in South Dakota corn was the largest single item in the diet of the pheasant. He listed the following seeds as being important as pheasant food: corn, wheat, barley, oats, and foxtail. From Michigan, Pirnie (1930) wrote, "The winter foods usually available . . . include the seeds

of many grasses and sedges, and such farm plants as ragweed and pigweed. When other food is scarce, pheasants work at teasel, sticktights, burdocks, and milkweed pods."

In Colorado, Burnett (1921) and Maxson (1921) found that pheasants fed during the winter on corn, oats, ragweed, bindweed, and wild oats.

Hicks (1935) observed the distribution and abundance of pheasants in Ohio to be rather closely linked with the corn crop. Dalke (1935) confirmed the importance of corn as pheasant food. He reported that in Michigan, one-third of the annual food of the pheasant is corn, including waste grain which remained in the field, corn dropped by pastured stock, corn carried into the woods by squirrels, and also that which was returned to the fields in the form of manure.

HABITS OF FEEDING

Feeding was usually done in the early morning and late afternoon, seldom at mid-day. In late fall the pheasants left cover at the hours of 6:00 to 8:30 a. m. and were back in shelter by 5:00 p. m. Because food was plentiful and readily available at that season less time was required for pheasants to obtain sufficient food than was the case later. As winter progressed the birds stayed at the roosting cover later in the morning before going out to feed, and on clear cold days they remained in the fields until after dark. On stormy and windy days the pheasants returned to loafing cover before noon and generally went to feed again for two to three hours in late afternoon.

There seemed to be very little fighting among the pheasants while they were feeding. Both sexes were usually found in the flocks of birds and there was no evidence to indicate that either sex fed more than the other during the winter. In early fall the pheasants were spread throughout the fields while feeding, but as colder weather set in they tended to group together to some extent. While feeding in the most severe weather the birds crowded quite close together in flocks of as many as 50 individuals.

Pheasants fed on manure whenever it was available in the fields. Because manure spreaders could not be used on the snow covered ground it was the common practice to haul the manure out in sleds and pile it in the fields until such a time as it could be spread. Pheasants flocked to these manure piles in large numbers where they fed until the manure froze so hard that the corn could not be taken out.

During the last half of February, when food was scarce, two flocks of 25 and 50 pheasants entered two different hog lots nearly every day to feed on corn with the hogs. In some instances these pheasants would not fly when the farmers approached or if they did fly away a short distance they soon returned.

Sometimes pheasants ranged considerable distances in order to feed. In the middle of a 90-acre plowed field from which the nearest shelter was about 80 rods away, one day in late fall a few birds, evidently in search of food, were observed to be traveling over the snow toward cornfields. Pheasants that roosted at the willow row, section 21, ranged from 40 to 80 rods to obtain food. Constant averages of bird counts made in various feeding areas indicated that many pheasants fed in the same fields day after day.

In general, feeding occurred twice a day and between the feeding periods the pheasants loafed in the fields or in more protective shelter. One feeding a day sufficed during the days of snowstorms. Whether or not the birds took any food during the middle of the day was a matter of conjecture. Leffingwell (1928) found that pheasants apparently fed twice a day, in the early morning and again before sunset, and he also suggested that although they might take food at other times than in morning or evening, here was no distinct search for it.

OTHER GAME BIRDS

Few Greater Prairie Chickens were on or near the area during the winter of 1935-36. Only one was known to have remained there all winter. It stayed in a 60-acre cornfield at the southwest corner of section 14. On November 13, 49 were reported by Harold Hove, but they were not seen again. On March 2, the author observed 18 on section 22. These birds probably were migrating back to the North.

In the fall of 1935 there were 36 European Partridges, *Perdix perdix perdix* (Linnaeus), on the area. The partridges were grouped into five coveys composed of six to nine birds. On sections 14, 15, 16, 21, and 22, respectively, were coveys of seven, six, nine, and seven partridges. In almost every instance the partridges were flushed from the tops of small knolls and only a few times from low ground or from groves. The ranging of coveys was rather restricted. From observations it seemed that a covey ranged over an area not exceeding one-eighth of a mile in radius.

The partridges appeared to be very hardy birds as evidenced by the winter survival of 35 of the 36 birds. One of the partridges was found dead on February 25. As the breast profile was 80 per cent of normal, that bird had not starved to death. No cause for its death was revealed during the examination of the dead partridge by the author and several other members of the Department of Zoology and Entomology.

In severe weather the partridges huddled closely together to conserve warmth. On several occasions the author flushed a covey of nine to observe that the depression left in the snow where they had roosted was smaller than that made by one pheasant.

Partridges and pheasants ranged in the same field but were never seen to feed together. No fighting between the two species was observed.

PHEASANT LOSSES

Before the hunting season in late fall it was estimated by the author that approximately 1,000 pheasants, or an average of one per 4.9 acres, resided on the eight section tract under observation. There was probably an influx of a few birds from Minnesota where the hunting season opened earlier than in Iowa. Farmers on both sides of the state line reported movements of birds into Iowa during the time that hunting was going on in Minnesota. Definite data on the exact numbers were not available. Although the author did not reach the area until after this movement was well under way he observed at least 10 crippled birds shortly after his arrival and before the Iowa hunting season opened. Probably these pheasants had been wounded by hunters across the state line.

After the hunting season the farmers of the area reported that 501 pheasants had been taken by hunters. Approximately 493 pheasants re-

mained to enter the winter. Of those, 423 were found on the south six sections (sections 14, 15, 16, 21, 22, and 23), most of the remainder on section 9, and only a few on section 10. It was estimated that there were about three hens to each cock at the beginning of the winter.

In attempts to account for the losses during the winter, daily searches were made for dead pheasants. The dead birds were collected, examined at least superficially, and those which had not been disturbed by scavengers were weighed. The weights of the dead hens varied from 1.50 to 2.25 pounds, and of the cocks from 1.50 to 4.00 pounds. The mean average weight of hens was 1.74 pounds and of cocks 2.64 pounds. Only one cock, found February 5, weighed as little as 1.50 pounds. The sternum of this male was prominent and poorly covered with flesh, and the remainder of the body was very emaciated. That cock was found in a straw stack (see Plate IV, fig. C). Without internal examination it was assumed that the bird in a weakened condition died from freezing. That cock was the only bird that showed advanced emaciation and probable starvation which accounted for 0.2 per cent of the population at the beginning of the winter. All other dead pheasants were in good flesh and quite plump.

Only one dead bird indicated probable predatory loss. A warm headless pheasant hen was found, but there were no signs nor tracks of a possible predator discerned in the vicinity. Snow was drifting lightly at the time and perhaps any signs were covered quickly. The probable predatory loss of pheasants was 0.2 per cent of the birds entering the winter.

The loss because of taking pheasants out of season was learned in part, at least. The illegal taking of 12 birds was reported to the investigator. That loss accounts for 2.3 per cent of the early winter population.

Fourteen dead pheasants showed early symptoms of pneumonia. Their mouths were bloody and showed more than normal amounts of mucus. As this loss was only 2.7 per cent of the total number of birds entering the winter, pneumonia did not seem to be of great importance as a lethal factor.

A large part of the loss of pheasants was attributed to freezing and choking which seemed to be very closely related. Two blizzards and three drift storms, each lasting one to two days and coupled with temperatures of zero to — 35° F., wreaked havoc among the pheasants. Birds, caught in drift storms and blizzards away from dense escape cover, almost invariably turned their tails to the wind and crouched on the snow. The body feathers of such unfortunate pheasants were ruffled and the driven snow was packed under the feathers. Body heat melted the snow and the severe cold caused the water to freeze and thus encase the birds in ice. Many of the ice-encased birds probably froze to death, for their bills and nostrils appeared to be clear of bloody or excessive exudates, and of the head not more than the eyes were covered with ice. Hence they probably did not choke to death. Perhaps the eyes of some of the birds were covered with ice before their death, and they were unable to find suitable protective cover. A few farmers on the area captured birds with ice-covered eyes and placed them in chicken houses until the ice melted. Upon liberation those pheasants soon flew away and appeared to have suffered but little from the experience. Some farmers reported the finding of pus in the eyes of the pheasants, evident only after the ice had melted from the eyes.

After those storms it was a common occurrence to find pheasants, heavily coated with ice and snow, that would not fly at the approach of the observer. Such birds ran a short distance and then stopped in a crouching position.

Following those five drift storms numerous pheasants were found with the bills or nostrils, and in some cases both of these parts, covered with ice (Plate VIII, fig. C and D). Probably in such cases the birds died of choking, although some of them were also encased in ice.

A few birds froze to death while roosting in the strawstacks. (Plate IV, fig. C, and Plate IX, fig. A and B).

In total, freezing or choking was considered responsible for the death of 137 pheasants, 27.70 per cent of the population remaining after the hunting season.

During blizzards and severe drifting some pheasants were covered so deeply with snow that they were not found until after the late winter thaws. The causes of death in those cases were not determined.

In early winter about 35 pheasants were observed to leave section 10 and cross the state line into Minnesota where they remained during the winter near a large thicket of first growth willows. These birds were not observed to return to the area in Iowa but the author saw them near the Minnesota willow thicket several times during the winter.

Although numerous reports were received of crows attacking and killing pheasants, the author did not observe crows molesting live pheasants. Probably such reports arose from observations of crows feeding on dead birds in the fields. Crows did act as scavengers. Without the crows it would have been more difficult to find some of the dead pheasants. Crows located even the partially snow-covered birds, plucked and scattered many of the feathers, and fed upon the carcasses (Plate IX, fig. C and D). The large dark patches of scattered feathers on the snow and occasional presence of crows made it possible to locate dead birds at considerable distances.

The known winter losses together with the percentages of the early winter population and causes of loss have been summarized in table 1.

TABLE 1. *Known winter losses*

Causes of loss	Number	Percentage of early winter population
Starvation	1	0.2
Predation	1	0.2
Illegal shooting	12	2.3
Pneumonia	14	2.7
Freezing or choking	137	27.7
Undetermined (snowed under)	38	7.6
Straying from area	35	7.5
Totals	238	48.2

Near the close of the winter quite accurate last counts of the pheasants totalled 246 birds as residing on the area. Those 246 birds added to the 238 birds known to have been lost during the winter accounted

for 484 of the early winter population. The most satisfactory and most nearly accurate counts of the pheasants in early winter totaled 493 birds as residing on the area. Thus nine birds were not accounted for in the course of the winter's observations. Perhaps a few or all of the nine birds unaccounted for were snowed under and not discerned after the late winter thaws, for some of the last dead birds picked up were so soiled that they were nearly invisible. As soon as the snow was nearly gone hogs were turned into several cornfields before the author had an opportunity to check the fields thoroughly.

As is discussed under the next heading, perhaps some birds strayed on to the area under investigation and others left in addition to the 35 known to have strayed from the area. Perhaps the nine birds unaccounted for, or several of them, were among those that strayed to neighboring fields.

At the close of winter the ratio between the sexes remained about the same as at the beginning of that season, namely three hens to one cock. Hence it did not appear that either sex was more resistant than the other to severe winter weather.

The populations of pheasants according to counts made by the investigator at several times during the period of investigation are summarized in table 2.

TABLE 2. *Pheasant populations of the area*

Pre-shooting population	1,000
Birds entering winter	493
Survivors	246

SURVIVAL AND LOSSES IN RELATION TO FOOD AND COVER

Concerning the importance of food and cover for pheasants Wight (1933) wrote, "On cold days pheasants, like many other animals, linger near or within roosting sites, and frequently do not leave the roost at all. Consequently their greatest comfort during a cold winter is derived from a dense roosting site near which a good food supply is available." On the area under investigation during the severe winter of 1935-'36 not only comfort but also survival depended on the proximity of food to dense cover.

No loss was known to occur near the willow row on the Elvebak Bros.' farm, section 22, nor at the willow row south of the Chris Walle farm, section 15. From neither place were birds required to range far for food. On the Elvebak farm seven birds depended throughout the winter largely on an acre of corn, with nearly half the ears unpicked, located just south of the willow row. On the Chris Walle farm the willow row was located just south of the farmyard, and in severe weather the pheasants fed in the hog lot with the hogs. The grove on the Walle farmstead offered protection from the wind as the birds went from the willow row to the hog lot to feed. The 18 or 20 birds seen at the willow row and hog lot were there only during the most severe weather. Probably they came from the flock of 34 pheasants present at the 60-acre field of corn in more open weather, because its reduced numbers, 14 or 16 in the most inclement weather, were then flushed close to the willow tree in the 60-acre field.

At the Lars Flo grove the early winter number of nine pheasants was reduced by one bird, or 11.1 per cent; and eight were left in late winter.

At the Erdol grove the early eight were reduced by four, or 50 per cent, that were picked up in a barren field nearby.

Mortality was low in the flock of the 60-acre hand-picked field of corn with a willow tree in the center, section 14. In the field and at some distance from the willow two dead pheasants were found, five dead birds were discovered in adjoining pastures and plowed fields, and three were poached. The early winter flock of 34 pheasants increased in late winter to 36 despite the loss of 10 members. The 10 dead birds added to the increase of two accounted for 12 new members joining the flock during the winter. Perhaps those 12 came from the slough on the Chris Walle farm, section 15, after the tall reeds had drifted full. Three-eighths of a mile of weedy fence lines, although full of snow, formed a sheltered path around the plowed field between the sloughs and willows of the Walle farmstead which at times were cover for some of the flock of the 60-acre cornfield. The 12 lost birds were about 26 per cent of the 46 (34 plus 12) sometime members of the flock. In the 60-acre cornfield pheasants did not have to range any great distance in feeding because the corn closely surrounded the willow tree, and while the birds fed in the corn the standing stalks protected them from the elements to some extent.

The next higher percentage of loss by death occurred in the 40-acre field of sweet corn, section 22, where 69 pheasants, about 39.5 per cent of the highest count of 175 birds, were lost. Fifty-nine dead pheasants were picked up in the corn, five were found in adjoining pastures and plowed fields, and five were taken illegally. Despite the lack of cover other than that afforded by the cornstalks, many pheasants remained in the sweet corn day and night. The closest denser cover was nearly 40 rods away but at no time were pheasants observed to move from the sweet corn to that cover. In late winter just before the thaws uncovered more food in the adjoining machine-picked fields 96 birds remained in the sweet corn. The loss by death of 69 added to the 96 remaining birds totalled 165 pheasants as sometime winter residents in the sweet corn. If 13 pheasants of the slough on the Walle farm, section 15, otherwise unaccounted for, came to the sweet corn field, the maximum resident number was 178 pheasants, three more than the high count of 175 birds seen at one time in the sweet corn. As the food became scarcer in the sweet corn near the end of the winter, and before the thaws uncovered corn of machine-picked fields, perhaps 13 birds not accounted for otherwise (178 minus 165 dead birds and those living in late winter) strayed off the area to hand-picked corn just south of section 22. In that corn pheasants were observed but not counted.

There was a loss of 10 birds, or 50 per cent, of the 20 pheasants entering the winter at the unmown sweet clover, section 16. Five of the lost birds were found dead in the sweet clover, two dead birds were located in an adjoining cornfield, one was found dead in a roadside ditch, and two were taken out of open season. After the sweet clover drifted full probably three of the remaining 10 birds were frozen in the nearby straw stack. The closest food available to those birds was an adjoining field of machine-picked corn at the north. After that corn drifted over the pheasants went to a hand-picked field about 30 rods south of the sweet clover. A deep snowdrift in the fence line running north and south gave some protection from the wind to the birds going between the clover and

the corn. After the sweet clover drifted full perhaps the remaining seven birds went from their usual feeding field of 12 acres of hand-picked corn to the willows in section 21, which still had some cover value.

A higher percentage of birds was lost in death from the flock that frequently sought shelter in the vacant grove, section 9. The grove was separated from a large field of hand-picked corn by a 50-foot strip of mown alfalfa. Across the road and west of the first cornfield lay an even larger acreage of hand-picked corn which offered additional food throughout the winter. Even while the birds fed in those fields they were protected from the adverse elements by the standing stalks. Of the flock wintering at the grove at least 39 perished in the most severe weather. Sixteen of the 39 dead pheasants were found in the three nearby cornfields, 15 were picked up in the adjoining mown alfalfa, five were gathered from the narrow strip of mown sweet clover between two nearby cornfields, and three in the road south of the fields. At the grove the daily counts in early winter averaged 35 birds and the numbers increased following heavy snowfall later in the winter to 75. The latest winter daily counts averaged 49 birds. In computing the percentage of loss the total number of birds that must be considered was 88, obtained by adding 39 that died and the 49 remaining. The probable gain of 53 birds over the early number of 35 was considered to be made up in part of 30 pheasants from the slough on the L. Olson farm, section 16, after the sedges and slough grass drifted full. Perhaps the additional 23 birds came from cornfields with slight cover of the section west of the vacant grove. The loss was 44.3 per cent of the total 88 pheasants.

A loss of 47 dead birds, about 44.2 per cent of 105 early residents, occurred in the flock which sought shelter at the willow thicket on the Olson Bros.' farm, section 21. Thirteen died in the willows, 19 in the cornfields, 13 in nearby pastures and plowed fields, and two were poached. The willows were isolated in a six-acre field of corn stubble, except for a grassy shallow ditch leading out toward the southwest. The closest available corn was about 100 feet from the thicket. After the first snow the ditch filled up and offered no shelter, even as a lane of travel from the willows to the cornfields. Hence the birds were entirely exposed to the weather as they ranged from the willows to feed. The late winter count of 46 birds plus the 47 dead birds accounted for 93 members of the flock. Perhaps seven pheasants came from the snow-filled sweet clover on section 16 to the willows to make the total of 112 sometime residents. That number, 112, minus 93 (living at the end of winter and those found dead) left 19 to be accounted for in other ways. Perhaps 10 dead birds found in the strawstack about 60 rods west of the willows were among the 19 otherwise missing. The remaining nine, perhaps, strayed to hand-picked corn about one-half mile west in section 20.

Because no dead birds were found in the slough on the L. Olson farm, section 16, and as there was a gain of 53 birds at the vacant grove, section 9, it was thought that the 30 birds of the slough went to the grove after the slough filled with snow. The vacant grove was close to the other side of the hand-picked cornfields in which the pheasants previously fed.

Four birds, 11.4 per cent, of the 35 birds entering the winter at the sloughs and willow row of the Chris Walle farm, section 15, were found dead in the vegetation of the sloughs. The cover value was not as good as the low percentage of loss would indicate, however, because the pheas-

ants left the slough early in the winter after it drifted full. It was assumed that six of the remaining 31 birds went to a strawstack where they were found frozen, 12 moved to the 60-acre cornfield on section 14, and the other 13 traveled south to the sweet corn field, section 22.

No pheasants roosted regularly at strawstacks and it was not known how many sought shelter in straw in inclement weather. Nineteen pheasants, the greatest loss in roosting cover, perished in three strawstacks. Ten pheasants, including the starved cock, were found at the strawstack west of the willows of Olson Bros.' farm, section 21, and probably they were former members of the flock that earlier found shelter in the willows. Six birds were found dead in the strawstack on the Iverson farm, section 22, and it was believed that those birds were from the 35 which started the winter at the slough on the Chris Walle farm, section 15. The other three birds were picked up at the strawstack just south of the sweet clover strip on the Harold Hove farm, section 16, and were no doubt part of the 20 pheasants that entered the winter in that clover.

SUMMARY

Pheasants were found in a variety of cover including bare ground, mown and grazed fields, unmown and ungrazed sloughs, weedy fencerows and ditches, farm crops, strawstacks, thickets and deciduous groves. Pheasants were not seen in evergreen groves of which none adjoined feeding grounds.

For escape cover pheasants used cornfields, willows, sloughs, strawstacks, tall sweet clover, and deciduous groves. No field was set off as a refuge for the pheasants during the hunting season. Perhaps bare ground was of refuge value.

Birds loafed in several types of cover: deciduous groves, willows, strawstacks, sloughs, weedy ditch banks and fence rows.

Pheasants were not observed to roost elsewhere than on the ground. In mild weather of late fall many pheasants roosted in cornfields, stubble fields, and sloughs, whereas, few birds roosted in willows, groves, and unmown sweet clover. During storms and cold winter weather many pheasants roosted in groves, willows, sloughs, unmown sweet clover and sweet corn whereas few roosted in more open cover.

The food of the pheasants was quite varied during the winter months. Listed in the order of importance, the following seeds were taken by the pheasants as food: field corn, sweet corn, oats, pigeon grass, corn (in manure), lesser ragweed, lamb's quarters, smartweed, soy beans, pigweed, and greater ragweed.

Usually the birds fed twice a day, in early morning and late afternoon, and seldom at mid-day. Between feeding periods the pheasants loafed in the fields or in more protective shelter.

Only one Greater Prairie Chicken stayed on the area during the winter. Thirty-six European Partridges entered the winter on the area, and only one succumbed during the winter. In almost every instance the partridges were flushed from the tops of small knolls.

Out of approximately 1,000 pheasants entering the winter, an average of one per 4.9 acres, 501 were reported as taken by hunters, and 493 remained to enter the winter. At the close of the winter's observations 246 pheasants were left on the area. A loss of 238 birds, 48.2 per cent, was

known to have occurred and only nine were considered to be unaccounted for.

Starvation and predation losses were negligible, and the poaching loss was low. The highest losses came from freezing and choking during severe weather.

Crows did not attack living pheasants but were scavengers on carcasses of dead pheasants.

Survival was highest in the flocks that roosted in dense cover of willows and groves adjacent to an available food supply that required little ranging to obtain. Survival was less in the flocks that roosted in dense cover but which were required to range over long distances to obtain food. Losses were highest in the flocks that roosted in open cover and that were forced to range some distance in feeding.

PLATE I

Diagram map of the area on which these studies were carried on. This map shows sections, farms, and tenants.

PLATE I


MINNESOTA
STATE LINE ROAD
IOWA
N
PLATE I
DIAGRAM MAP SHOWING SECTIONS, FARMS, & OPERATORS OF RANGE 25, TOWNSHIP 100, WINNEBAGO COUNTY, IOWA
LEGEND
K County Trunk Road
10 Center & Number of Section
80 Acres JOHN VERLO
80 Acres JULIUS JELLE
54 Acres PETE SELVIG
9
160 Acres PETE SELVIG
80 Acres ALBIN CARLSON
80 Acres THORE BRAADLAND
107 Acres ARNOLD FALEIDE
107 Acres ARNOLD FALEIDE
10
160 Acres CARL JUHL
160 Acres DAVID SKUTTLE
160 Acres LEONARD OLSON
160 Acres LEWIS RYGH
16
240 Acres HAROLD HOVE
80 Acres RUSSELL SIME
120 Acres JOHN VERLO
160 Acres MARIE WALLE
15
200 Acres LARS FLO
160 Acres CHRIS WALLE
120 Acres EINAR ALMBERG
120 Acres CLEMENT FURE
80 Acres ED. WALLE
14
80 Acres CHRIS WALLE
160 Acres NESJE BROS.
80 Acres L. FOSNESS
240 Acres OLSON BROS.
80 Acres MORRIS ERDAL
21
160 Acres FRED LAMPING
160 Acres ANDREW HESTNESS
40 Acres MORRIS ERDAL
40 Acres LARS FLO
320 Acres LAWRENCE IVERSON
22
160 Acres ELVEBAK BROS.
80 Acres CHRIS OLSON
40 Acres CLEMENT FURE
120 Acres NESJE BROS.
200 Acres ELMER SEIM
23
160 Acres CHRISTENSON BROS.
120 Acres CHRIS CHRISTENSON

PLATE II

Farm crops and game cover map. This map shows the potential cover on the area, and illustrates the food and cover relationships found there.

PLATE II

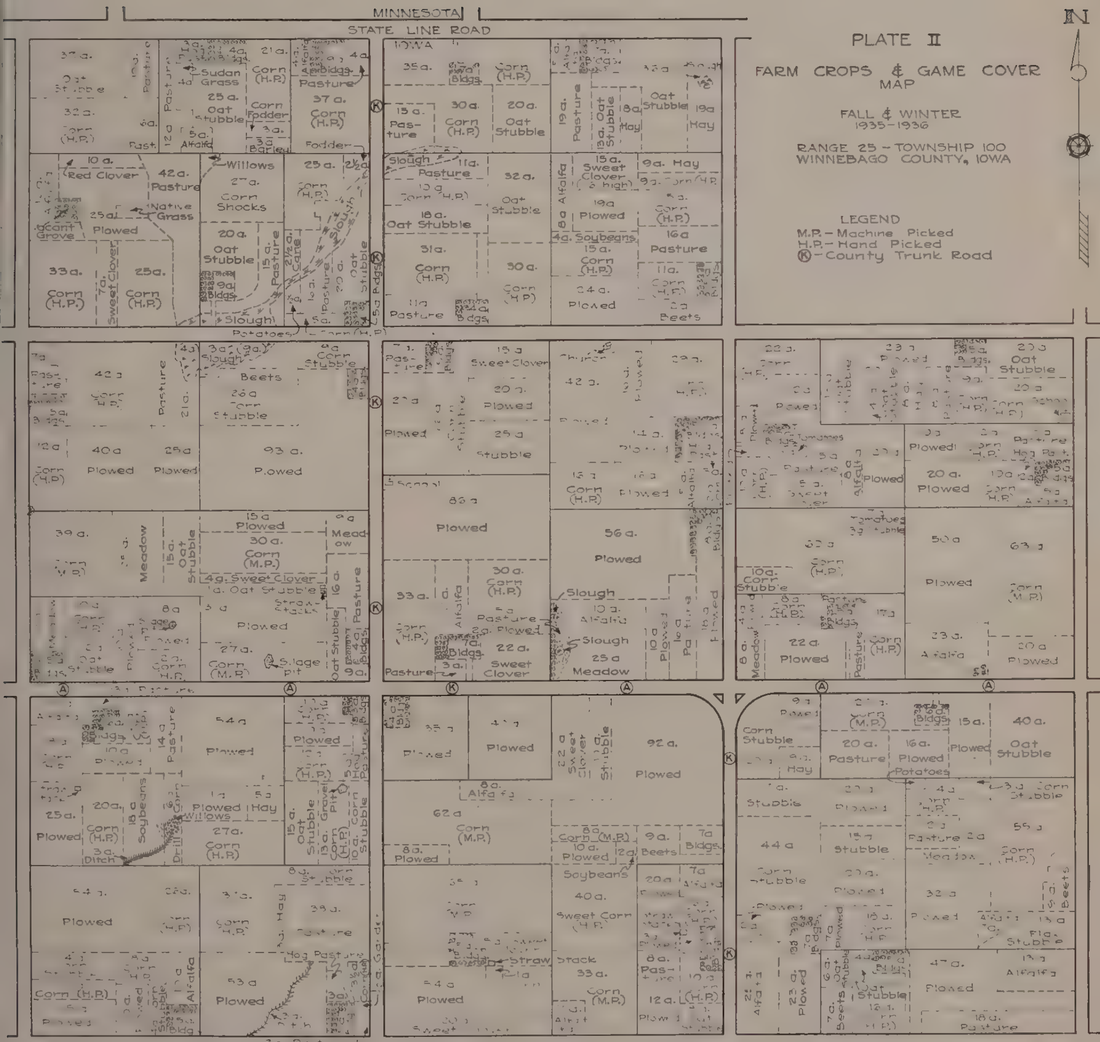
MINNESOTA
STATE LINE ROAD
IOWA
N
PLATE II
FARM CROPS & GAME COVER MAP
FALL & WINTER 1935–1936
RANGE 25 – TOWNSHIP 100
WINNEBAGO COUNTY, IOWA
LEGEND
M.P. – Machine Picked
H.P. – Hand Picked
Ⓚ – County Trunk Road

PLATE III

Fig. A. Sweet clover before drifting occurred. Note density of cover.

Fig. B. Sweet clover drifted over. Note lack of cover.

Fig. C. Tall reeds (*Phragmites communis*). These were used as cover before they were completely drifted over.

Fig. D. Contrast between hand-picked sweet corn and machine-picked field corn. Note lack of cover in machine-picked field in foreground.

PLATE IV

Fig. A. Vacant grove, section 9. Note density of cover and the windbreak offered here.

Fig. B. Vacant grove, section 9.

Fig. C. Cock burrowed in straw. This bird was found dead.

Fig. D. Willow row, section 15. This grove occasionally harbored 20 pheasants.

PLATE III

PLATE IV

PLATE V

Fig. A. Willow row, section 14. This row harbored no pheasants

Fig. B. Willow located in the middle of a 60-acre field of hand-picked corn, section 14. This willow provided cover for 14 to 34 pheasants during the winter.

Fig. C. Willow row east of Elvebak farmstead section 22. Seven pheasants sought shelter here during the winter.

Fig. D. Willow clump, section 21, from distance. Note lack of food or cover in surrounding area.

PLATE VI

Fig. A. Willow clump, section 21, before drifting started. As many as 125 pheasants were found here early in winter. Note the apparent excellent cover afforded by these willows.

Fig. B. Same willow clump, one-half drifted over.

Fig. C. Same clump, three-fourths drifted over. Note scarcity of cover.

Fig. D. Same clump completely drifted over. Note lack of cover. This series of pictures illustrates what happened to cover during the winter.

PLATE V

PLATE VI

PLATE VII

Fig. A. Willow row along west side of Walle's slough, section 15.
Fig. B. Burdock. Note pheasant tracks in foreground.
Fig. C. Evidence of pheasant feeding. Note kernels missing from ear.
Fig. D. Burdock. Note the mats of burs.

PLATE VII

PLATE VIII

Fig. A. Hand-picked cornfield. Note ears left on stalks. Such hills were the source of much food for pheasants.

Fig. B. General view of a hand-picked field of corn. This was taken in late February. Note the cover still offered by stalks.

Fig. C. Dead pheasant cock. Note ice over eyes and bill. The bird was found in this condition and position.

Fig. D. Dead pheasants. This picture was taken after the ice had melted. Note how the bills were opened. When found, the bills were full of ice.

PLATE IX

Fig. A. Pheasant hen found frozen in strawstack.

Fig. B. Pheasants found frozen in straw. Three hens and two cocks were found here.

Fig. C. Evidence of crow work on frozen birds. The feathers scattered in this way greatly facilitated the finding of dead birds.

Fig. D. Evidence of crow work.

PLATE VIII

PLATE IX

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BUCKLING LOADS FOR EDGE LOADED CLAMPED PLATES[1]

DONALD H. ROCK

From the Department of Mathematics, Iowa State College

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Although the problem of the stability of the clamped square plate has been solved by various individuals, the general results for the rectangular plate have not been handled. This investigation is concerned with the extension to this latter case and to the case in which the plate is reinforced by the addition of two ribs.

The method employed is the well-known energy approximation method of Ritz (1) and Timoshenko (2).

THE ENERGY METHOD

The differential equation for the bending of the plate is replaced by the equivalent problem of minimizing the expression for the edge-loading N as a function of the strain energy of bending and the work of the external forces. If we denote by V the strain energy of bending:

$$(1) \qquad V = \frac{D}{2}\int\int\left\{(\nabla^2 w)^2 - 2(1-\nu)\left[\frac{\partial^2 w}{\partial x^2}\frac{\partial^2 w}{\partial y^2} - \left(\frac{\partial^2 w}{\partial x \partial y}\right)^2\right]\right\}dxdy$$

where $D = \dfrac{EI}{1-\nu^2}$, and by T the work of the external forces:

$$(2) \qquad T = \frac{N}{2}\int\int(\nabla w)^2 dxdy,$$

where N is the force per unit edge length, then the condition for the existence of equilibrium is $T = V$. Hence we may write for N:

$$(3) \quad N = \frac{D\displaystyle\int\int\left\{(\nabla^2 w)^2 - 2(1-\nu)\left[\frac{\partial^2 w}{\partial x^2}\frac{\partial^2 w}{\partial y^2} - \left(\frac{\partial^2 w}{\partial x \partial y}\right)^2\right]\right\}dxdy}{\displaystyle\int\int(\nabla w)^2 dxdy}.$$

In order to obtain the critical value for N by Ritz's method we assume a form for w:

$$(4) \qquad w_n = \Sigma c_n \phi_n$$

---

[1] A thesis submitted to the Graduate Faculty of Iowa State College in partial fulfillment of requirements for the degree of Master of Science, June, 1937. This investigation was supported by the Industrial Science Research Fund.

where the ϕ_n satisfy the boundary conditions $\phi = 0$ and $\frac{\partial \phi}{\partial n} = 0$ on the contour, and the c_n are undetermined parameters. The problem is reduced then to minimizing equation (3) with respect to the parameters c_n after the above form of w is inserted. This yields n linear homogeneous equations from which the desired solution for N is obtained.

In order to facilitate the work of computation, w is chosen in the following way:

$$(5) \qquad w_n = \Sigma\Sigma A_{mn} u_m(x) v_n(y)$$

where

$$(6) \qquad u_m(x) = \frac{\cosh k_m \cos k_m \dfrac{x}{a} - \cos k_m \cosh k_m \dfrac{x}{a}}{\sqrt{a} \cdot \sqrt{\cosh^2 k_m + \cos^2 k_m}},$$

with v_n a similar function in y with a replaced by b (2a is the length, 2b the width, of the plate). The functions u (x) and v (y) are selected as above because they represent a symmetric form for the deflection of the plate. The k's are determined from the equation:

$$(7) \qquad \tanh k + \tan k = 0.$$

It will be seen on inspection that these functions satisfy the boundary conditions, and further that they are orthogonal in their respective regions. That is:

$$(8) \qquad \int_{-a}^{a} u_m(x) u_p(x)\, dx = 0 \ (m \neq p), \quad \int_{-b}^{b} v_n(y) v_q(y)\, dy = 0 \ (n \neq q).$$

If now the minimization of equation (3) with respect to the A_{mn} is carried out, certain type integrals occur which are listed in table 1.

TABLE 1. *Type integrals*

$$\int_{-a}^{a} u_m(x) u_p(x)\, dx = \begin{cases} 0, & m \neq p \\ 1, & m = p \end{cases}, \qquad \int_{-a}^{a} u_m''(x) u_p''(x)\, dx = \begin{cases} 0, & m \neq p \\ \dfrac{k_m^4}{a^4}, & m = p \end{cases},$$

$$\omega_{mp}(x) = \int_{-a}^{a} u_m''(x) u_p(x)\, dx = \left. \frac{a^4[u_m'''(x) u_p''(x) - u_m''(x) u_p'''(x)]}{k_m^4 - k_p^4} \right|_{-a}^{a},$$

$$\omega_{pm}(x) = \int_{-a}^{a} u_m(x) u_p''(x)\, dx = \omega_{mp}(x),$$

$$\omega_{mm}(x) = \int_{-a}^{a} u_m''(x) u_m(x)\, dx$$

$$= \frac{-k_m^2 (\cosh^2 k_m \cos^2 k_m) + 2k_m \cosh^2 k_m \cos^2 k_m \tanh k_m}{a^2 (\cosh^2 k_m + \cos^2 k_m)},$$

$$\alpha_{mp}(x) = \int_{-a}^{a} u_m'(x) u_p'(x) \, dx = -\omega_{mp}(x), \qquad \omega_{mo}(x) = \omega_{om}(x) = 0,$$

$$\alpha_{mm}(x) = \int_{-a}^{a} u_m'(x) u_m'(x) \, dx = -\omega_{mm}(x), \qquad \alpha_{mo}(x) = \alpha_{om}(x) = 0,$$

(These apply also to $v(y)$ with a replaced by b.)

THE RECTANGULAR CLAMPED PLATE

Let the plate be bounded by $x = \pm a$, $y = \pm b$. If the notation of table 1 is employed, the set of equations to be solved after application of the minimizing conditions is:

$$(7) \qquad A_{mn}\left(\frac{k_m^4}{a^4} + \frac{k_m^4}{b^4}\right) + 2 \sum_p \sum_q A_{pq}\omega_{mp}(x)\,\omega_{nq}(y) + \frac{\lambda}{a^2}\left[\sum_p A_{pn}\omega_{mp}(x) + \sum_q A_{mq}\omega_{nq}(y)\right] = 0,$$

where $\lambda = \frac{a^2 N}{D}$. Here m and n take all even positive integral values.

For the second approximation for which m and n take the values 2 and 4 the following results were obtained for various values of the plate ratio $\beta = \frac{b}{a}$:

(8)

β	1.0	1.5	2.0	3.0	4.0
λ	13.20	10.28	9.80	8.75	8.54

THE REINFORCED CLAMPED PLATE

As before we let the plate be bounded by $x = \pm a$, $y = \pm b$. At $x = \pm \frac{a}{3}$ we insert two stiffeners. The equation of equilibrium is now:

$$(9) \qquad V + \Sigma V_i = T + \Sigma T_i,$$

where V_i is the strain energy of bending the ith rib, and T_i is the work done during buckling by the compressive force P_i acting on the ith rib. These are (3):

$$(10) \qquad V_i = \frac{B_i}{2}\int_{-b}^{b}\left(\frac{\partial^2 w}{\partial y^2}\right)^2_{x=a/3} dy, \qquad T_i = \frac{P_i}{2}\int_{-b}^{b}\left(\frac{\partial w}{\partial y}\right)^2_{x=a/3} dy,$$

where B_i is the rigidity of the ith rib.

The equation for N is then:

$$(11) \qquad \frac{N}{D} = \frac{\iint\left\{(\nabla^2 w)^2 - 2(1-\nu)\left[\frac{\partial^2 w}{\partial x^2}\frac{\partial^2 w}{\partial y^2} - \left(\frac{\partial^2 w}{\partial x \partial y}\right)^2\right]\right\}dxdy + 2\gamma\int_{-b}^{b}\left(\frac{\partial^2 w}{\partial y^2}\right)^2_{x=a/3} dy}{\iint(\nabla w)^2 dxdy + 2\delta\int_{-b}^{b}\left(\frac{\partial w}{\partial y}\right)^2_{x=a/3} dy}$$

where $\delta = \dfrac{P}{aN}$ and $\gamma = \dfrac{B}{aD}$. The ratio δ measures, in the case concerned, the ratio of the width of the stiffener to the length of the plate, while γ is the ratio of the rigidities.

The minimization of equation (11) with respect to the A_{mn} leads to the system of equations:

$$(12) \qquad A_{mn}\left(\frac{k_m^4}{a^4} + \frac{k_n^4}{b^4}\right) + 2\sum_p\sum_q A_{pq}\omega_{mp}(x)\,\omega_{nq}(y)$$
$$+ \frac{k_n^4}{b^4} a\gamma u_m(a/3)\sum_p A_{pn}u_p(a/3) + \frac{\lambda}{a^2}\Bigg[\sum_p A_{pn}\omega_{mp}(x) + \sum_q A_{mq}\omega_{nq}(y)$$
$$+ a\delta u_m(a/3)\sum_p\sum_q A_{pq}u_p(a/3)\,\omega_{nq}(y)\Bigg] = 0.$$

The λ's resulting from the second approximation for various values of β, δ, and γ are given in table 2.

TABLE 2. *The λ's resulting from the second approximation*

	$\gamma = 2$		$\gamma = 5$		$\gamma = 10$	
β	$\delta = 0.1$	$\delta = 0.2$	$\delta = 0.1$	$\delta = 0.2$	$\delta = 0.1$	$\delta = 0.2$
1.0	19.88	19.25	30.57	27.94	33.01	29.25
1.5	36.37	36.12	37.69	37.68	37.93	37.93
2.0	45.48	45.48		45.50		

CONCLUSIONS

The buckling load for the square plate has been caluculated by various investigators. Their results are shown below.

Sezawa (4)	$\lambda = 5.61$
Taylor (5)	$\lambda > 5.30$
Weinstein (6)	$5.304 < \lambda < 5.312$
Trefftz (7)	$5.30 < \lambda < 5.32$
Faxen (8)	$\lambda = 5.305$

The result obtained in this investigation is 13.20 which, when multiplied by the conversion factor $\frac{4}{\pi^2}$, becomes 5.35. This is seen to be in good agreement with the previously obtained results. Timoshenko has shown the value of λ for the infinitely long clamped plate to be 8.388. The values of λ in table 1 approach this value very rapidly.

The author wishes to make acknowledgement to Dr. D. L. Holl for his guidance in the course of this investigation.

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A FLORA OF WINNESHIEK AND ALLAMAKEE COUNTIES AND CLAYTON COUNTY IN THE VICINITY OF McGREGOR

WILLIAM L. TOLSTEAD

From the Department of Botany, Iowa State College

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During the summer of 1933 an opportunity was afforded to collect and study the flora of the extreme northeastern part of Iowa[1]. This flora is significant in its relation to the climate, the geological and glacial history of the region, and the resultant peculiar erosional topography and the soils. Though much of the original plant cover has been destroyed during 75 years of occupancy by the white man, and many exotic species have been introduced, some representative spots remain along railroads, in unpastured meadows and in woods throughout the region.

The collecting was done in Winneshiek and Allamakee counties, and in the vicinity of McGregor in Clayton County. These three counties lie in the Mississippi River Valley on the Minnesota border in the northeastern corner of Iowa and together form a rectangular area embracing approximately 2,300 square miles.

PHYSICAL ENVIRONMENT

PHYSIOGRAPHY AND GEOLOGY

Since the Cretaceous period the region has been subject to alternate rising and base-leveling as indicated by the Dodgeville and Lancaster peneplains and the rock bottoms of the ancient valleys which are one to two hundred feet below present-day valley levels. As thus evidenced, the drop from the highest to the lowest point in the region was greater in the past than it is at present. On account of the filling of the valleys, the present elevation ranges from 1300 feet above sea level in northern Winneshiek County to 670 feet near McGregor. This fall of 630 feet occurring over a distance of 50 miles results in relatively swift flowing rivers which in the past were probably swifter than they are now because of inequalities in elevation greater than those of today. Water erosion has cut deep, narrow valleys, leaving broad, rolling, upland prairie divides.

Three major drainage systems, flowing eastward to the Mississippi River, have developed. The Upper Iowa River, with a number of small tributaries, drains the northern parts of Winneshiek and Allamakee counties; the Yellow River, which rises in the southeastern part of Winneshiek County, drains the southern part of Allamakee County; and the Turkey River drains only the southwestern quarter of Winneshiek County. Along the eastern border of Allamakee County are several minor creeks which flow directly into the Mississippi River.

On either side of the Mississippi Valley are narrow strips of steep and high rugged cliffs. Here the rock formations are exposed. They are chiefly limestones and dolomites, with a few formations of sandstones and

[1] Through the grant of a research fellowship at Iowa State College during 1933 and 1934, the study was extended.

shales of minor importance. They are of the Cambrian, Ordovician, Silurian and Devonian systems, the Cambrian rocks being exposed only along the Mississippi River Valley and the Devonian rocks in western and southwestern parts of Winneshiek County.

During the Pleistocene, the area now included in Winneshiek and Allamakee counties was more or less affected by three periods of glaciation and loess deposit. Glaciation was not severe in the eastern two-thirds of the region, where slight evidence leads early workers to call the region "non-glaciated." However, the work of Kay and Apfel (1929) has revealed that probably all of the two counties, with the possible exception of the highest hilltops, was covered by the Nebraskan glacier, and since this early Pleistocene time the eastern two-thirds of the region probably has been free from glaciation.

The second and third ice sheets to enter the region were the Kansan and the Iowan substage of the Wisconsin glacial age (Kay and Leighton, 1933). These glaciers spread over the western part of Winneshiek County, leaving behind deposits of till, filling the valleys, and cutting down the steep hillsides. Thus the relatively level prairie topography typical of western Winneshiek County contrasts sharply with the less glaciated, hilly lands to the east.

The flood waters from the melting Kansan and early Wisconsin (Iowan) glaciers deposited great banks of sand in the river valleys where they remain today in the form of river terraces. During interglacial times the region was overlaid with a mantle of loess. Loess deposits are especially noticeable on the Kansan and "non-glaciated" areas.

SOILS

The soils of Winneshiek and Allamakee counties are derived largely from loess and glacial till. These original materials have undergone changes since their deposition. Organic matter has accumulated; oxidation and leaching of soluble materials has taken place near the surface; and the materials have been subject to varying degrees of drainage. According to Marbut (1923) these processes have affected greater changes upon the soils as they exist today than have the original differences in the character of the parent material.

The soils of the region are divided by Benton and Russell (1927) into light and dark colored soils, depending upon the amount of accumulated organic matter. The light colored soils are found in the hilly areas where good drainage prevails and where the microclimate favors a vegetation dominated by trees. Here decomposition of organic matter has been rapid. The dark colored soils developed on the rolling prairie lands where climate gave rise to a vegetation dominated by tall grasses. In these grass lands slow decomposition of accumulated organic matter has resulted in dark soils. Benton and Russell recognized two groups of dark soils; those which developed under poor drainage and those which developed under good drainage.

CLIMATE

Winneshiek and Allamakee counties are located in the cooler parts of Iowa, and have a shorter growing season, and a rainfall above the average for the state. The average annual precipitation at Decorah for a period

of 40 years prior to 1930 was 32.96 inches, 1.08 inches above the state average. For this period the following comparisons may be made: The rainfall at Keokuk, in southeastern Iowa, was 33.67 inches, and at Sioux City, in northwestern Iowa, 23.57 inches. The greatest rainfall occurs during the growing season and the average rainfall for the spring and summer months is 20.4 inches. May and June are normally the wettest months, and January the driest. The wettest season occurred in 1902 with a precipitation of 43.22 inches, the driest year was in 1933 with a precipitation of 16.75 inches.

Cold waves from the north bring winter temperatures far below zero. The lowest recorded temperature is — 37° F., the highest is 108° F. The average annual temperature at Decorah is 45.7° F., at Keokuk 52.1° F., and at Sioux City 48° F.

The average growing season without frost is shorter than in any other section of the state. At Decorah its duration is 140 days, at Keokuk 170 days, and at Sioux City 148 days. The latest killing frost ever recorded at Decorah occurred June 8, and the earliest in the fall on August 30. The average date of the last spring frost is May 10 and the average date for the first frost in the fall is September 28.

THE FLORA

EARLY COLLECTIONS AND RECORDS

The first collections of plants of Winneshiek and Allamakee counties were made during the last quarter of the nineteenth century by local people, among whom may be mentioned E. W. Holway and Herbert Goddard of Decorah, and Mrs. H. C. Carter of Hesper. Their collections were reported in annotated lists by Arthur (1876).

The earliest work limited to the flora of this region is that of Macbride (1894) on the forest trees of Allamakee County. In 1896 Fitzpatrick and Barsch made a trip down the Upper Iowa River. The lists of Fitzpatrick, which also include a large number of records from other collectors, were published in 1898. During the summer of 1902 and 1903 Shimek made extensive collections throughout Winneshiek County. Lists of his collections, as well as those of Thomas E. Savage and others, were published in 1906. As shown by specimens in the herbarium at Iowa State College, subsequent collections have been made by B. Shimek, L. H. Pammel, R. I. Cratty, Ada Hayden, C. M. King and others. Publications pertaining to the flora of the region have been made by Pammel (1905), Shimek (1904), Rosendahl and Butters (1929), and Cratty (1896 and 1898).

PROCEDURE IN PRESENT WORK

The present collection was begun during the spring and summer of 1933. The major portion of the collection was done in the Upper Iowa River Valley, in the Mississippi River Valley near New Albin, Lansing, and McGregor, and on the prairie ridge from Ridgeway to Ossian. During the summers from 1934 to 1937 collecting trips were made to various parts of the region. The specimens were identified and compared with those in the herbarium at Iowa State College. Since the collection at the University of Iowa was not open for consultation while the present work was under way, it was impossible to inspect material which might have ap-

plied to such a study as this. The mosses were verified by Dr. H. S. Conard, and certain doubtful specimens of seed plants were sent to Gray Herbarium and Arnold Arboretum for verification.

In naming the plants the international rules of botanical nomenclature were followed. In naming the mosses, the usage of Grout (1903), (1933-1937) was adopted; for the grasses, Hitchcock (1935); and for the other plants, the nomenclature of Gray's Manual (1908), except where revisions require changes. The plant list is arranged as to families according to the Engler and Prantl system, but the genera and species are listed alphabetically.

One set of specimens collected in this survey is deposited in the herbarium at Iowa State College, and another at Luther College, Decorah, Iowa.

ANNOTATED SYSTEMATIC LIST

Division I. BRYOPHYTA

Musci (The Mosses)

POLYTRICHACEAE (Hair-cap Family)

CATHARINEA ANGUSTATA Brid.
Collected in open woods on south hillside near Upper Dam. Growing in sandy soil.

POLYTRICHUM JUNIPERINUM Willd.
In open woods at Decorah in oak-hickory woods and at Eldorado in open woods on sandy ridge. Common throughout the region.

DICRANACEAE (Dicranum Family)

CERATODON PURPUREUS Hedw. Brid.
Common throughout the region. A xeric form occurred on rocky areas on hilltop at Decorah and a mesic form in ditches along roads.

DICRANUM SCOPARIUM (L.) Hedw.
Collected near Decorah on south, shady hillside.

GRIMMIACEAE (Grimmia Family)

LEUCOBRYUM GLAUCUM (L.) Schimp.
On south hillside in deep woods at Lower Dam in Upper Iowa River Valley. Growing on soil.

TORTULACEAE (Tortula Family)

BARBULA FALLAX Hedw.
Collected near Decorah in linden-maple woods.

DIDYMODON RUBELLUS (Hoffm.) B. & X.
Growing on calcareous rocks in linden-maple woods on south hillside at Decorah.

FUNARIACEAE (Orthotrichum Family)

FUNARIA HYGROMETRICA (L.) Sibth.
On moist sandstone cliff on shady hillside near Upper Dam in Upper Iowa River Valley.

PHYSCOMITRIUM ACUMINATUM Bruch. and Schimp.
On mud deposit in pasture after floods in Upper Iowa River Valley.
PHYSCOMITRIUM Hookeri Hemp.
Collected with the above species.

TIMMIACEAE (Timmia Family)

TIMMIA CUCULLATA Michx.
Timmia megapolitana Hedw.
Collected near Decorah in Twin Springs Park. Growing on soil in moist, shady location.

BARTRAMIACEAE (Bartramia Family)

BARTRAMIA OEDERI (Gunn.) Swtz.
Plagiopus Oederi (Gunn.) Limpr.
Collected in linden-maple woods near Decorah. Growing on humus soil on calcareous rocks.
BARTRAMIA POMIFORMIS (L.) Hedw.
Collected at Decorah on moist, shady bank. Growing on humus soil over calcareous rocks.

BRYACEAE (Bryum Family)

MNIUM CUSPIDATUM (L.) Leyss.
In linden-maple woods on moist soil in Glenwood township in Winneshiek County.
MNIUM MEDIUM B. & S.
In linden-maple woods near Decorah in swampy areas.
RHODOBRYUM ROSEUM (Weis.) Schreb.
In fruit on humus soil over calcareous talus on south, shaded, moist hillside in open woods near Decorah.

HYPNACEAE (Hypnum Family)

ANOMODON ATTENUATUS (Schreb.) Hueben.
On limestone rocks in linden-maple woods at Decorah.
ANOMODON MINOR (P. Beauv.) Lindberg.
Growing on log in linden-maple woods in Canoe Creek Valley.
ANOMODON ROSTRATUS (Hedw.) Schimp.
Growing on limestone rock talus along Dugway Road at Decorah in moist shaly location.
AMBLYSTEGIUM IRRIGUUM (Wils.) B. & S.
Hygroamblystegium irriguum (Wils.) Loeske
Along edge of creek on rocks in Canoe Creek Valley.
AMBLYSTEGIUM VARIUM (Hedw.) Lindb.
Collected in open woods near Eldorado on sandy soil on hilltop above river. Associated with *Polytrichum juniperinum*.
BRACHYTHECIUM ACUMINATUM (Hedw.) Kindb.
Chamberlainia acuminata (Hedw.) Grout
Growing on old stump in woods in Pleasant Twp.
BRACHYTHECIUM OXYCLADON (Brid.) J. & S.
On calcareous rocks on south hillside west of Decorah.
BRACHYTHECIUM OXYCLADON DENTATUM (L. & J.) Grout
On old log in woods near Decorah.

CALLIERGON SCHREBERI Willd.
Calliergonella Schreberi (Willd., Br. & Sch.) Grout
In woods near Decorah.
CLIMACIUM AMERICANUM Brid.
In swamp areas at small springs, in Upper Iowa River Valley. Commonly forming a dense mat of vegetation on calcareous rocks.
ENTODON CLADORRHIZANS (Hedw.) C. Muell.
Growing on old log in woods near mouth of Canoe Creek.
HYLOCOMIUM SPLENDENS (Hedw.) Grout.
Hylocomium proliferum (L.) Lindb.
On limestone talus on moist, shady hillsides in linden-maple woods near Upper Dam.
HYPNUM CURVIFOLIUM Hedw.
HYPNUM HALDANIANUM Grev.
Heterophyllum Haldanianum Grev.
Above two species were collected in linden-maple woods near Decorah.
PLATYGYRIUM REPENS (Brid.) B. & S.
On log in woods.
RHYTIDIUM RUGOSUM (Ehrh.) Kindb.
On limestone cliff on south, shady hillside at Bluffton.
RHYTIDIADELPHUS TRIQUETUS L. (Hedw.) Warnst.
Hylocomium triquetum (L.) B. & S.
South hillside, linden-maple woods east of Decorah.

Division II. PTERIDOPHYTA

POLYPODIACEAE (Fern Family)

ADIANTUM PEDATUM L. Maidenhair Fern.
Common in linden-maple woods throughout the area.
ATHYRIUM ACROSTICHOIDES (Sw.) Diels. Silvery spleenwort. See Rhodora 21:173.
Asplenium acrostichoides Sw.
In linden-maple woods at mouth of Canoe Creek and two miles south of Lansing. Rare.
ATHYRIUM ANGUSTUM (Willd.) Presl. Upland Lady Fern. See Rhodora 19:190. 1917.
Asplenium Filix-femina (L.) Bernh. in part
Common in woods, especially in the upland oak woods.
CAMPTOSORUS RHIZOPHYLLUS (L.) Link. Walking Fern.
On calcareous rocks and cliffs where humus and mosses have accumulated. Found only in linden-maple woods on protected hillsides.
CHEILANTHES FEEI Moore. Slender Lip Fern.
On limestone cliffs on sunny hillsides in Allamakee County in the Upper Iowa River Valley. Rare.
CRYPTOGRAMMA STELLERI (Gmel.) Prantl. American Rock-brake.
In moist crevices on limestone cliffs in linden-maple woods. Collected at Decorah and Lower Dam.
CYSTOPTERIS BULBIFERA (L.) Bernh. Bladder Fern.
Filix bulbifera (L.) Underwood.

Common in linden-maple woods, growing in humus soil overlaying limestone rocks, especially on talus slopes below limestone cliffs on south, protected hillsides.

CYSTOPTERIS FRAGILIS (L.) Bernh. Brittle or Fragile Fern.

Filix fragilis (L.) Gilib.

In moist woods; not as common as the above species.

ONOCLEA SENSIBILIS L. Sensitive Fern.

In swamp north of Freeport, and in upland woods near Bluffton and in Mississippi River Valley woods at Marquette.

PELLAEA GLABELLA Mett. Cliff Brake.

See Rydberg, Flora of the Prairies and Plains of Central North America.

Common on limestone cliffs on both shady and sunny hillsides.

POLYPODIUM VIRGINIANUM L. Common Polypody.

See Rydberg, Flora of the Prairies and Plains of Central North America. See Rhodora 24: 141. 1922.

Collected only in the Mississippi River Valley on stones and cliffs on moist, wooded hillsides.

POLYSTICHUM ACROSTICHOIDES (Michx.) Schott. Christmas-fern.

In linden-maple woods two miles south of Lansing on Mississippi Valley hillsides. No fruiting fronds were seen when collected in July. Rare.

PTERETIS NODULOSA (Michx.) Nieuwl. Ostrich Fern.

Not *Onoclea Struthiopteris* (L.) Hoff. of Gray's manual. See Am. Midland Nat. 4: 334, 1916; Rhodora 17: 161-164, 1915; and Rhodora 21: 178, 1919.

On alluvial soil in open woods along Canoe Creek.

PTERIDIUM LATIUSCULUM (Desv.) Maxon. Bracken Fern.

Pteris aquilina L. of Gray's manual. See Am. Fern Journal 9: 43. 1919.

Common in upland oak woods. Especially abundant in recently cut-over or burned-over land.

THELYPTERIS GOLDIANA (Hook.) Nieuwl. Goldie's Fern.

Aspidium Goldianum Hook. of Gray's Manual

Dryopteris Goldiana (Hook.) Gray

The generic name Thelypteris, in place of either *Aspidium* or *Dryopteris*, is adopted here. See Nieuwland Am. Midland Nat. 1: 226, 1910; and to it is united the genus *Phegopteris*. See Weatherby, Rhodora, 21: 179. 1919.

In linden-maple woods along Canoe Creek in Winneshiek County, and on west hillside of Mississippi River Valley two miles south of Lansing. Rare.

THELYPTERIS HEXAGONOPTERA (Michx.) Weatherby. Broad Beech-fern.

Phegopteris hexagonoptera (Michx.) Fee.

In linden-maple woods at mouth of Yellow River in Allamakee County. Rare.

THELYPTERIS ROBERTIANA (Hoffm.) Slosson. Scented Oak-fern.

Phegopteris Robertiana (Hoffm.) A. Br.

Found at Decorah and Bluffton on humus soil overlying calcareous rocks on shady locations.

THELYPTERIS PALUSTRIS Schott. var. PUBESCENS (Lawson) Fernald. Marsh Shield Fern
Dryopteris Thelypteris (L.) A. Gray
Aspidium Thelypteris Sw.
On prairie lowlands one mile east of Ridgeway along the Milwaukee Railroad. Rare.

THELYPTERIS SPINULOSA (O. F. Muller) Nieuwl. Spinulose Shield-Fern.
Aspidium spinulosum (O. F. Muller) Sw.
In woods of Canoe Creek Valley, especially around old decayed logs.

WOODSIA ILVENSIS (L.) R. Br. Rusty Woodsia.
Collected in Winneshiek County by Dr. John L. Lewis, I.S.C. 24, 529.

WOODSIA OBTUSA (Spreng.) Torr. Blunt-lobed Woodsia.
At Upper Dam in Upper Iowa River Valley in moist humus soil on wooded hillside.

WOODSIA SCOPULINA D. C. Eaton. Rocky Mountain Woodsia.
At Decorah on calcareous cliff on shady south hillside. This is the only specimen of this species in I. S. C. Herbarium. Collections previously reported were found to be *Woodsia oregana* D. C. Eaton.

OSMUNDACEAE (Flowering Fern Family)

OSMUNDA CLAYTONIANA L. Clayton's Fern
Common in large colonies in woods throughout the region.

OPHIOGLOSSACEAE (Adder's Tongue Family)

BOTRYCHIUM TERNATUM (Thunb.) Sw., var. INTERMEDIUM D. C. Eaton. Ternate Grape Fern.
On prairie along Milwaukee railroad one mile east of Ridgeway and on sandy river terrace one mile south of Freeport.

BOTRYCHIUM VIRGINIANUM (L.) Sw. Virginia Grape Fern.
Scattered in deep woods throughout the region.

SALVINIACEAE (Salvinia Family)

AZOLLA CAROLINIANA Willd. Carolina Azolla.
I. S. C. Herbarium 16,661. Collected at Lansing by Charlotte M. King, Aug. 19, 1901.

EQUISETACEAE (Horsetail Family)

EQUISTUM ARVENSE L. Field Horsetail.
Common in fields and waste places.

EQUISETUM LIMOSUM L. Swamp Horsetail. See Rhodora 32: 45. 1921.
Equisetum fluviatile L.
In muddy slough one mile south of Lansing.

EQUISETUM HYEMALE L., var. ROBUSTUM (A. Br.) A. A. Eaton. Scouring Rush.
On banks of Upper Iowa River and sloughs in Mississippi River Valley.

LYCOPODIACEAE (Club Moss Family)

LYCOPODIUM COMPLANATUM L. Trailing Christmas-green.
I. S. C. Herbarium 104,201. Collected by C. Trower, June 4, 1925, at McGregor.

LYCOPODIUM LUCIDULUM Michx. Shining Club-moss.
I. S. C. Herbarium 113,162. Collected in Allamakee County by O. C. Schultz in July, 1924; Ada Hayden, Sept. 13, 1937.

SELAGINELLACEAE (Selaginella Family)

SELAGINELLA RUPESTRIS (L.) Spring. Rock Selaginella.
On sandy hillside of river terrace in shady locations; four miles west of New Albin in Upper Iowa River Valley.

Division III. SPERMATOPHYTA

Subdivision I. Gymnospermae

TAXACEAE (Yew Family)

TAXUS CANADENSIS Marsh. American Yew.
Common in moist woods on calcareous cliffs and talus.

PINACEAE (Pine Family)

ABIES BALSAMEA (L.) Mill. Balsam Fir.
On steep limestone cliffs on south, shady hillside across river from village of Bluffton. A large grove of trees about one-half mile long.

JUNIPERUS COMMUNIS L. Creeping Juniper.
On rocky, sunny hillsides in Upper Iowa River Valley. Common locally.

JUNIPERUS VIRGINIANA L. Red Cedar.
On limestone cliffs and rocky hillsides throughout the Upper Iowa River Valley. Often coming up on areas from which the oak and hickory trees have been cut. More common than *Juniperus communis*.

PINUS STROBUS L. White Pine.
Growing along Upper Iowa River on calcareous cliffs and well-drained, rocky, shallow soils.

Several species of exotic gymnosperms are grown and planted in lawns. Among them are:

LARIX LARICINA (Du Roi) Koch. American Larch.
LARIX DECIDUA Mill. European Larch.
PICEA ABIES (L.) Karst. Norway Spruce.
PICEA CANADENSIS (Mill.) BSP. Canadian Spruce.
PINUS MONTANA Mill. Mountain Pine.
PINUS SYLVESTRIS L. Scotch Pine.
THUJA OCCIDENTALIS L. American Arbor-vitae.
PSEUDOTSUGA TAXIFOLIA Brit. Douglas Fir.

Subdivision II. Angiospermae

Class 1. MONOCOTYLEDONEAE

TYPHACEAE (Cat-tail Family)

TYPHA LATIFOLIA L. Broad-leaved Cat-tail.
Common in swampy areas, especially in the Mississippi River Valley; occasionally on upland prairie swamps and one of the first plants to invade a newly formed pond or low, moist spot.

SPARGANIACEAE (Bur-reed Family)

SPARGANIUM EURYCARPUM Engelm. Broad-fruited Bur-reed.
Common in swamps and edges of sloughs in Mississippi River Valley.

ALISMACEAE (Water-plantain Family)

ALISMA SUBCORDATUM Raf. American Water-plantain.
See Arkiv. f. Bot. 24A[7]: 19. 1932.
Alisma Plantago-aquatica of Am. Auth. in part, not L.
Along shores of sloughs and in swamps in Mississippi River Valley.

SAGITTARIA LATIFOLIA L. Broad-leaved Arrowhead.
Common in swamps, sloughs, and along shores of rivers throughout the region. A narrow leaved form was found at Fort Atkinson.

GRAMINEAE (Grass Family)

TRIBE FESTUCEAE

BROMUS CILIATUS L. Ciliated Brome Grass.
I. S. C. Herbarium 96,484. Collected by L. H. Pammel at McGregor in July, 1919.

BROMUS INERMIS Leyss. Smooth Brome Grass.
Common weedy plant along roadsides and waste places.

BROMUS KALMII A. Gray. Kalm's Brome Grass.
Collected at McGregor in upland woods; Allamakee County, seven miles south of New Albin.

BROMUS LATIGLUMIS (Shear) Hitchc. Broad-glumed Brome Grass.
In moist woods in Upper Iowa River Valley, in alluvial soil. Not common.

BROMUS PURGANS L. Woodland Brome Grass.
Frequent in linden-maple woods near Decorah.

BROMUS TECTORUM L. Downy Brome Grass.
Very common weedy plant in disturbed areas.

DACTYLIS GLOMERATA L. Orchard Grass.
Common weed in lawns and waste places. European.

ERAGROSTIS CILIANENSIS (All.) Link. Stinkgrass. Candy Grass.
A weedy annual species in disturbed areas.

ERAGROSTIS FRANKII C. A. Meyer. Frank's Love-grass.
On banks of small creek near New Albin.

ERAGROSTIS HYPNOIDES (Lam.) BSP. Smooth Creeping Love-grass.
Along banks of dried sloughs in Mississippi River Valley.

ERAGROSTIS PECTINACEA (Michx.) Nees. Purple Love-grass.
In sandy soil on river terrace in Upper Iowa River Valley near New Albin and Freeport.

ERAGROSTIS PILOSA (L.) Beauv. Small Tufted Love-grass.
Collected on sandy river terrace near Freeport.

ERAGROSTIS SPECTABILIS (Pursh) Steud. Purple Love-grass.
On sandy river terrace one-half mile south of Freeport.

FESTUCA ELATIOR L.
I. S. C. Herbarium 141,359. Collected at McGregor by Jesse Fults, July 1, 1934.

FESTUCA OBTUSA Spreng. Nodding Fescue.
Festuca nutans Spreng.
In linden-maple woods at Decorah and McGregor.

GLYCERIA GRANDIS S. Wats. American Mannagrass.
In swamp south of Lansing in Mississippi River Valley.

GLYCERIA STRIATA (Lam.) Hitchc. Fowl Mannagrass.
In swamp near Lower Dam.

MELICA NITENS (Scribn.) Nutt. Three Flowered Melic Grass.
In open woods on rocky hillside in Hanover Township in Allamakee County. Rare.

PHRAGMITES COMMUNIS Trin. Common Reed Grass.
Found at Lansing in lowlands along slough.

POA COMPRESSA L. Canada Bluegrass.
In lawns at Decorah.

POA PRATENSIS L. Kentucky Bluegrass.
Common dominant grass throughout the region.

TRIBE HORDEAE

AGROPYRON REPENS (L.) Beauv. Quackgrass.
Common grass throughout the region; often becoming a pest in fields.

AGROPYRON PAUCIFLORUM (Schwein.) Hitchc. Slender Wheat Grass.
A. tenerum Vasey.
Found at Decorah and McGregor.

AGROPYRON SMITHII Rydb.
Occurs in well drained, sandy areas along railroad tracks at Decorah and McGregor.

ELYMUS CANADENSIS L. Canada Wild Rye.
Collected at Decorah in open woods on rocky calcareous soil and near Union City in valley woods.

ELYMUS CANADENSIS L. var. ROBUSTUS (Scribn. and Smith.) Mackenz. and Bush. Robust Canada Wild Rye.
Common on upland prairies at Calmar, Ridgeway and in Mississippi Valley on sandy soil near New Albin.

ELYMUS VIRGINICUS L. Virginia Wild Rye.
In woods at Lower Dam in Upper Iowa River Valley.

ELYMUS VILLOSUS Muhl. Slender Wild Rye.
Elymus striatus of Am. Auth. not Willd.
Common in valley woods.

HORDEUM JUBATUM L. Foxtail Barley.
Common weedy plant along roadsides, gardens, and lawns.

HYSTRIX PATULA Moench. Bottlebrush.
Collected in open woods at Decorah, at mouth of Yellow River in Allamakee County, and at McGregor.

TRIBE AVENEAE

AVENA FATUA L. Wild Oat Grass.
In oat fields of the region.

DANTHONIA SPICATA (L.) Beauv. Poverty Oat-grass.
Collected in upland oak woods at McGregor.

KOELERIA CRISTATA (L.) Pers. Junegrass.
Common grass on prairie grasslands throughout the region.

SPHENOPHOLIS INTERMEDIA (Rydb.) Rydb. Slender Wedge-grass.
In linden-maple woods on hillside at McGregor.

TRIBE AGROSTIDEAE

AGROSTIS ALBA L. Redtop.
Common introduced grass growing throughout region in fields, roadsides, lawns, and waste places.

AGROSTIS HIEMALIS (Walt.) BSP. Ticklegrass.
On sandy soil on river terrace one-half mile south of Freeport, and on St. Peter sandstone debris at McGregor.

AGROSTIS PERENNANS (Walt.) Tuckerm. Autumn Bent.
I. S. C. Herbarium 96,871. Collected by L. H. Pammel near McGregor August 20, 1920.

ARISTIDA BASIRAMEA Engelm. Forked Triple-awned Grass.
On sandy soil in Mississippi River Valley and on sandy river terraces in Upper Iowa River Valley near New Albin.

ARISTIDA OLIGANTHA Michx. Prairie Three-awned Grass.
On dunes and in Upper Iowa River Valley near New Albin.

BRACHYELYTRUM ERECTUM (Schreb.) Beauv.
I. S. C. Herbarium 141,341. Collected by Jesse Fults near McGregor, July 1, 1934.

CALAMAGROSTIS CANADENSIS (Michx.) Beauv. Blue-joint.
In swamp meadow one mile south of Lansing in Mississippi River Valley.

CINNA ARUNDINACEA (Michx.) Beauv. Stout Woodreed.
Along banks of small stream near Fort Atkinson.

MUHLENBERGIA CUSPIDATA (Torr.) Rydb. Plains Muhly.
Collected on top of wind swept hill along highway between Decorah and Calmar. Shallow soil above limestone rock.

MUHLENBERGIA FOLIOSA (Roem. and Schult.) Trin. Foliose Muhly.
Along the Milwaukee railroad at New Albin, Allamakee County, Sept. 10, 1934.

MUHLENBERGIA MEXICANA (L.) Trin. Wirestem Muhly.
In ditch along road on prairie one mile west of Calmar.

MUHLENBERGIA RACEMOSA (Michx.) BSP. Marsh Muhly.
Common on prairie at Calmar and Ridgeway.

MUHLENBERGIA SCHREBERI Gmel. Nimblewill. Schreber's Muhly.
In open woods in Upper Iowa River Valley four miles west of New Albin.

MUHLENBERGIA SYLVATICA Torr. Wood Muhly.
I. S. C. Herbarium 141,369. Collected by Jess Fults near McGregor, July 1, 1934.

ORYZOPSIS RACEMOSA (J. E. Smith) Ricker. Ricegrass.
In linden-maple woods near Bluffton and McGregor.

PHLEUM PRATENSE L. Timothy.
Common escape from cultivation, in fields, roadsides, and waste places.

SPOROBOLUS ASPER (Michx.) Kunth. Long-leaved Dropseed.
On sandy soil along Milwaukee Railroad at New Albin.

SPOROBOLUS CRYPTANDRUS (Torr.) A. Gray. Sand Dropseed.
Common on sandy river terraces in Upper Iowa River Valley in Allamakee County and in the Mississippi Valley.

SPOROBOLUS HETEROLEPIS A. Gray. Prairie Dropseed.
On prairie along Milwaukee Railroad one mile west of Calmar; growing with Big and Little Blue Stem and Needle-grass.

SPOROBOLUS VAGINIFLORUS (Torr.) Wood. Sheathed Dropseed.
A weedy grass on newly disturbed soil along roads near Ridgeway and New Albin.

STIPA SPARTEA Trin. Porcupine Grass.
Common grass on prairies between Calmar and Ridgeway along Milwaukee Railroad. Growing with *Koeleria cristata, Andropogon furcatus* and *Andropogon scoparius.*

TRIBE CHLORIDEAE

BOUTELOUA CURTIPENDULA (Michx.) Torr. Side-oats Grama Grass.
Common grass on well drained soils on steep valley hillsides and prairie ridges.

BOUTELOUA HIRSUTA Lag. Hairy Grama Grass.
On sandy river terraces in the Upper Iowa River Valley. In certain locations a dominant grass.

SPARTINA PECTINATA Link. Prairie Cordgrass.
Spartina Michauxiana Hitchc.
In swampy lands on upland prairies and Mississippi River Valley. Common grass of low moist lands.

TRIBE PHALARIDEAE

PHALARIS ARUNDINACEA L. Reed Canary Grass.
In swampy land on prairie near Ridgeway and in Mississippi River Valley swamps.

TRIBE ORYZEAE

LEERSIA LENTICULARIS Michx. Catchfly Grass.
I. S. C. Herbarium 107,945. Collected by L. H. Pammel along Yellow River in Allamakee County, August 10, 1922.

LEERSIA ORYZOIDES (L.) Swartz. Rice Cutgrass.
Common in swamp one mile north of Freeport and above the Lower Dam in the Upper Iowa River Valley.

LEERSIA VIRGINICA Willd. White Grass.
In swamp north of Freeport. Not common.

TRIBE ZIZANIEAE

ZIZANIA AQUATICA L. Annual Wild Rice.
In swampy land along slough at New Albin.

TRIBE PANICEAE

CENCHRUS PAUCIFLORUS Benth. Sandbur.
Common in fields and waste places in sandy soil.

DIGITARIA SANGUINALIS (L.) Scop. Crabgrass.
Common weed in fields, gardens, and waste places.

ECHINOCHLOA CRUSGALLI (L.) Beauv.
Common weed in fields and gardens.

LEPTOLOMA COGNATUM (Schult.) Chase. Fall Witch Grass.
A weedy plant on sandy soil of river terraces in Upper Iowa River Valley.

PANICUM CAPILLARE L. Witch-grass.
A weedy plant in gardens and waste places.

PANICUM DICHOTOMIFLORUM Michx. Fall Panicum.
I. S. C. Herbarium 9,537. Collected in Winneshiek County by G. W. Carver.

PANICUM HUACHUCAE Ashe. Hairy Panic Grass.
Collected on prairie near Ossian.

PANICUM IMPLICATUM Scribn. Slender-stemmed Panic Grass.
Rare in open woods in Upper Iowa River Valley four miles west of New Albin.

PANICUM LATIFOLIUM L. Broad-leaved Panic Grass.
At Lower Dam and McGregor in open woods.

PANICUM LEIBERGII (Vasey) Scribn. Leiberg's Panic Grass.
Common on prairies between Calmar and Ridgeway.

PANICUM PERLONGUM Nash. Long-stalked Panic Grass.
Collected near New Albin on sandy soil along Milwaukee Railroad. Rare.

PANICUM DEPAUPERATUM Muhl.
Collected near McGregor on Pike's Peak; in sandy soil and in open woods.

PANICUM PRAECOCIUS Hitchc. and Chase. Early Branching Panic Grass.
On prairie near Calmar.

PANICUM SCRIBNERIANUM Nash. Scribner's Panic Grass.
Common on prairies between Calmar and Ridgeway.

PANICUM VIRGATUM L. Switch Grass.
Common on prairie grasslands throughout the region. Dominant on dune area on Upper Iowa River terrace.

PASPALUM STRAMINEUM Nash. Straw-colored Paspalum.
In sandy soil on river terrace in Upper Iowa River Valley in Allamakee County.

SETARIA LUTESCENS (Weigel.) F. T. Hubbard. Yellow Foxtail.
Setaria glauca (L.) Beauv. Rh. 18: 232. 1916.

SETARIA VIRIDIS (L.) Beauv. Green Foxtail Grass.
Both of the above species of *Setaria* are common weedy plants in disturbed areas throughout the region.

SETARIA VERTICILLATA (L.) Beauv. Foxtail Grass.
Collected in garden at Decorah. Not common.

TRIBE ANDROPOGONEAE

ANDROPOGON FURCATUS Muhl. Big Blue Stem Grass.
See Hitchcock, A. S., Man. of Grasses of the U. S. Dept. Agri., Mis. Pub. 200: 490. 1935; and Fernald, M. L., and L. Griscom, Rh. 37: 146. 1935.
Andropogon provincialis Lam.
Very common, dominant grass on the prairie lands throughout the region.

ANDROPOGON SCOPARIUS Michx. Little Blue Stem Grass.
On prairie ridges and steep hillsides throughout the region. Commonly associated with *Bouteloua curtipendula.*
SORGHASTRUM NUTANS (L.) Nash. Indian Grass.
On prairies in habitats similar to *Andropogon furcatus,* but not as abundant.

CYPERACEAE (Sedge Family)

CAREX ALBURSINA Sheldon. White Bear Sedge.
In herbaceous layer of linden-maple woods. Common.
CAREX ASSINIBOINENSIS W. Boott. Assinioboia Sedge.
Collected in linden-maple woods at Decorah.
CAREX BLANDA Dewey. Woodland Sedge.
Common in linden-maple woods throughout the region.
CAREX BEBBII Olney. Bebb's Sedge.
I. S. C. Herbarium 87,562. Collected by Goddard in Winneshiek County, August 1, 1899.
CAREX BREVIOR (Dewey) Mackenzie. Fescue Sedge.
See Am. Midl. Nat. 4: 235. 1915.
Carex molesta Mack.
Carex festucacea var. *brevior* (Dewey) Fernald.
Collected on prairie near Calmar.
CAREX CEPHALOPHORA Muhl. Oval-headed Sedge.
Collected in Pulpit Rock Park, Decorah, in open woods.
CAREX CONVOLUTA Mackenzie. Convolute Sedge.
See Bull. Torr. Bot. Club 43: 428. 1916.
Carex rosea of auth. not Schkuhr.
I. S. C. Herbarium 43,938. Collected by Holway at Decorah, May 29, 1881; I. S. C. Herbarium 77,988. Collected by Pammel at Postville, June 22, 1918.
CAREX EBURNEA Boott. Bristle-leaved Sedge.
On south hillside in open woods along Dugway road at Decorah. On moist, cool rocks and rocky soil.
CAREX GRAVIDA Bailey. Heavy Sedge.
I. S. C. Herbarium 71,474. Collected in Winneshiek County by Herbert Goddard.
CAREX GRISEA Wahl. Gray Sedge.
In linden-maple woods at Decorah.
CAREX HIRTIFOLIA Mackenzie.
I. S. C. Herbarium 140,983. Collected by Holway on wooded hillside at Decorah, June 2, 1901.
CAREX HYSTERICINA Muhl. Porcupine Sedge.
Common in swampy land, especially at springs.
CAREX LAEVICONICA Dewey.
In swamps in Mississippi River Valley near New Albin.
CAREX LANUGINOSA Michx. Woolly Sedge.
In swampy places at Lower Dam and on prairie near Calmar.
CAREX SPRENGELII Dewey. Long-beaked Sedge.
Carex longirostris Torr.
See Mackenzie, K. K., North Am. Flora 18: 298. 1935.
Common in linden-maple woods at Decorah and Lower Dam.

CAREX LUPULINA Muhl. Hop Sedge.
In swampy land in Mississippi River Valley south of Lansing and at McGregor.

CAREX MUSKINGUMENSIS Schwein.
In swamps at New Albin and McGregor in Mississippi Valley.

CAREX NORMALIS Mackenzie.
I. S. C. Herbarium 71,586. Collected by Goddard at Decorah, July 31, 1899.

CAREX PEDUNCULATA Muhl. Long-stalked Sedge.
In woods on south hillside near Highlandville in Winneshiek County.

CAREX PENNSYLVANICA Lam. Pennsylvania Sedge.
Common in open woods and pastures, especially in oak-hickory woods on north hillsides.

CAREX RETRORSA Schwein.
Collected in Mississippi River Valley in swamp near Lansing.

CAREX ROSEA Schkuhr. Stellate Sedge.
Common in linden-maple woods.

CAREX STIPATA Muhl. Awl-fruited Sedge.
Common in swamps and low places on prairie.

CAREX STRICTA Lam. Tussock Sedge.
In swamp seven miles south of New Albin in Allamakee County, by Ada Hayden, Sept. 13, 1937.

CAREX TRICHOCARPA Muhl.
Collected at Lansing in Mississippi Valley swamp.

CAREX TYPHINA Michx.
In valley woods chiefly of soft sugar maples and American elm across river from McGregor.

CAREX VULPINOIDEA Michx. Fox Sedge.
In swamp at Lower Dam and on prairies.

CYPERUS INFLEXUS Muhl. Awned Cyperus.
Cyperus aristatus Boeckl. in part, not Rottb.
Along shores of small creek in sandy soil at Fort Atkinson and Lansing.

CYPERUS ERYTHRORHIZOS Muhl. Red-rooted Cyperus.
Two miles north of New Albin on edge of dried up slough.

CYPERUS ESCULENTUS L. Yellow Nut-grass
In swamp near Lansing.

CYPERUS FILICULMIS Vahl. Slender Cyperus.
Common on sandy soil, especially on the river terraces.

CYPERUS RIVULARIS Kunth. Shining Cyperus.
Along creek in sandy soil near Fort Atkinson.

CYPERUS SCHWEINITZII Torr. Schweinitz's Cyperus.
Common in sandy soil and often found growing with *C. filiculmis* on the sandy river terraces in Upper Iowa River Valley.

CYPERUS STRIGOSUS L. Straw-colored Cyperus.
In moist soil along creek at Fort Atkinson.

ELEOCHARIS ACICULARIS (L.) R. & S. Least Spike-rush.
Growing in dense mats on edge of slough two miles north of Lansing.

ELEOCHARIS CALVA Torr. Creeping Spike-rush.
E. glaucescens of Am. auth. See Rh. 31: 68-40. 1929.
Along edge of Upper Iowa River at Bluffton.

ELEOCHARIS OBTUSA (Willd.) Schultes. Blunt Spike-rush.
Collected by Ada Hayden on edge of pond in Upper Iowa River Valley 10 miles east of New Albin, on Sept. 13, 1937.

SCIRPUS ATROVIRENS Muhl. Dark-green Bulrush.
On prairies along Milwaukee Railroad near Calmar.

SCIRPUS CYPERINUS (L.) Kunth., var. PELIUS Fernald. Wood Grass.
I. S. C. Herbarium 88,183. Collected by H. Goddard in Winneshiek County, July 30, 1899.

SCIRPUS PEDICELLATUS Fernald. Pedicellate Wool Grass.
On lowland prairie along Milwaukee Railroad near Calmar.

SCIRPUS VALIDUS Vahl. Great American Bulrush.

ARACEAE (Arum Family)

ACORUS CALAMUS L. Sweet Flag.
Growing in swamps one mile north of Freeport and two miles south of Lansing.

ARISAEMA DRACONTIUM (L.) Schott. Dragon Root.
Collected only at mouth of Canoe Creek in linden-maple woods.

ARISAEMA TRIPHYLLUM (L.) Schott. Indian Turnip, Jack-in-the-Pulpit.
Common in linden-maple woods throughout entire region.

SYMPLOCARPUS FOETIDUS (L.) Nutt. Skunk Cabbage.
Found in Winneshiek County one mile north of Freeport at outlet of spring, and in a similar habitat in Canoe Creek Valley. Flowers very early before the leaves appear. The leaves die about the middle of the summer.

COMMELINACEAE (Spiderwort Family)

TRADESCANTIA BRACTEATA Small. Long Bracted Spiderwort.
Collected at Decorah on rocky, sunny hillside. Rare.

TRADESCANTIA CANALICULATA Raf. Reflexed Spiderwort.
See Contr. Am. Arb. 9: 74. 1935.
Tradescantia reflexa Raf.
Common on sandy soils at Decorah and on river terraces at Upper dam.

JUNCACEAE (Rush Family)

JUNCUS MACER S. F. Gray. Yard Rush.
See Jour. Bot. 68: 366. 1930. (*Juncus tenuis* Willd.).
In hard soil in paths at Lansing.

LUZULA CAMPESTRIS (L.) DC., var. MULTIFLORA (Ehrh.) Celak. Wood Rush.
Luzula multiflora (Ehrh.) Les.
In moist woodlands on south hillside at Upper Dam in Upper Iowa River Valley. Rare.

LILIACEAE (Lily Family)

ALLIUM CANADENSE L. Meadow Garlic.
In open woods in Pleasant Township in Winneshiek County.

ALLIUM CERNUUM Roth. Nodding Wild Onion.

Rare in cool, shady hillsides in linden-maple woods at Bluffton and Highlandville, on humus soil on talus limestone rock.

ALLIUM STELLATUM Ker. Prairie Wild Onion.

I. S. C. Herbarium 43,759. Collected by Holway at Decorah, July 20, 1879.

ALLIUM TRICOCCUM Ait. Wild Leek.

Common in linden-maple and elm-ash woods throughout the region.

ASPARAGUS OFFICINALIS L. Asparagus.

Common escape from cultivation.

ERYTHRONIUM ALBIDUM Nutt. White Dog-Toothed Violet.

Common in moist elm-ash woods and often extending up into the wooded hillsides.

ERYTHRONIUM AMERICANUM Ker. Yellow Dog-Toothed Violet.

At Canoe Creek in elm-ash woods. Not as colonial as *Erythronium albidum*, occurring in small groups of only several plants. Rare.

LILIUM MICHIGANENSE Farw. Western Turk's Cap Lily.

Lilium superbum of auth. Not L.

See Bull. Torrey Bot. Club, 42: 353-354.

On moist prairie lands west of Ridgeway and on edge of swamp one mile north of Freeport. Becoming rare.

LILUM PHILADELPHICUM L., var. ANDINUM (Nutt.) Ker.

Collected on sunny hillside two miles southeast of Decorah and on prairie near Ossian along Milwaukee Railroad.

MAIANTHEMUM CANADENSE Desf. False Lily-of-the-Valley.

At Bluffton and Decorah in linden-maple woods in humus soil on calcareous rocks or talus. Not common.

OAKESIA SESSILIFOLIA (L.) Wats. Sessile-leaved Bellwort.

Rare in upland woods of black and white oaks at Conover Station.

POLYGONATUM BIFLORUM (Walt.) Ell. Hairy Solomon's Seal.

Rare in linden-maple woods at Bluffton.

POLYGONATUM COMMUTATUM (R. & S.) Dietr. Solomon's Seal.

Common in open woods and on prairie near Calmar.

SMILACINA RACEMOSA (L.) Desf. False Solomon's Seal.

Common in upland oak woods.

SMILACINA STELLATA (L.) Desf. Star-flowered Solomon's Seal.

Collected at Decorah in linden-maple woods and oak-hickory woods. Not as common as the above species but occurs in more xeric habitats.

SMILAX HERBACEA L. Carrion-flower.

Occasionally found in linden-maple woods.

SMILAX HISPIDA Muhl. Hispid Greenbrier.

Collected in thicket at Upper Dam.

SMILAX HERBACEA var. LASIONEURON (Hook.) A.DC. Tendril bearing upright smilax.

Smilax lasioneuron (Hook.) Rydb.

See Rydberg, Flora of Prairies and Plains.

Common along edges of fields, thickets, and open woods.

TRILLIUM CERNUUM L. Nodding Wake-robin.

In open woods on railroad right-of-way at Conover Station in moist, humus soil.

TRILLIUM GLEASONI Fernald. Drooping Wake-robin.
See Rhodora 34:21. 1932.
Trillium declinatum (Gray) Gleason not Raf.
Common in linden-maple woods throughout the region.

TRILLIUM NIVALE Riddell. Snow Trillium or Early Wake Robin.
Common in open woods in the valleys. The first spring flower.

UVULARIA GRANDIFLORA J. E. Smith. Large-flowered Bellwort.
Common in moist linden-maple woods.

ZIGADENUS ELEGANS Pursh.
See Rhodora 37:256. 1935.
Z. chloranthus Richards.
On east hillside two miles southeast of Decorah. In full sun with prairie grasses.

DIOSCOREACEAE (Yam Family)

DIOSCOREA VILLOSA L. Yam.
See Rhodora 20:48. 1919.
In open woods and thickets at Lower Dam.

AMARYLLIDACEAE (Amaryllis Family)

HYPOXIS HIRSUTA (L.) Coville. Star Grass.
Common on prairies and in open woods.

IRIDACEAE (Iris Family)

IRIS VIRGINICA L. Large Blue Flag.
See Anderson, Ann. Mo. Bot. Gard. 15:241-332. 1928.
Iris versicolor of Gray's Manual in part.
In swamps and on edges of ponds in the Mississippi and Upper Iowa River Valleys.

SISRINCHIUM CAMPESTRE Bicknell. Blue-eyed Grass.
Common on prairies and in open woods.

ORCHIDACEAE (Orchis Family)

CALOPOGON PULCHELLUS (Sw.) R. Br.
I. S. C. Herbarium 44,425. Collected by E. W. Holway at Freeport, June 20, 1880.

CYPRIPEDIUM CANDIDUM Muhl. Small White Lady's Slipper.
I. S. C. Herbarium 44,425. Collected by E. W. Holway in Winneshiek County, May 29, 1881.

CYPRIPEDIUM PARVIFLORUM Salisb. Small Yellow Lady's Slipper.
In swamp along railroad near Ridgeway and north of Cresco in Howard County.

CYPRIPEDIUM PARVIFLORUM Salisb. var. PUBESCENS (Willd.) Knight. Large Yellow Lady's Slipper.
Collected near Upper Dam in Upper Iowa River Valley and near Decorah in linden-maple woods.

EPIPACTIS PUBESCENS (Willd.) A. A. Eaton. Rattlesnake Plantain.
Collected in Allamakee County four miles west of New Albin in linden-maple woods.

HABENARIA BRACTEATA (Willd.) R. Br. Long-bracted Orchis.
In open woods on south hillside along Dugway road at Decorah.

HABENARIA HOOKERI Torr. Hooker's Orchis.
In upland oak woods three miles east of Decorah. Rare.
HABENARIA LEUCOPHAEA (Nutt.) Gray. Prairie White-fringed Orchis.
On prairies near Ridgeway in low land in soil with much humus and where the habitat has not been disturbed by deposit or washing of flood waters.
LIPARIS LILIIFOLIA (L.) Richard. Twayblade.
In rich upland oak woods three miles east of Decorah.
ORCHIS SPECTABILIS L. Showy Orchis.
Collected in woods at Upper Dam and in Canoe Creek Valley.
SPIRANTHES CERNUA (L.) Richard. Ladies' Tresses.
On lowland prairie along Milwaukee Railroad near Ridgeway. Swampy habitat.

Class 2. DICOTYLDONEAE

SALICACEAE (Willow Family)

POPULUS ALBA L. White or Silver-leaf Poplar.
Introduced tree from Europe. Commonly planted in yards and occasionally found as an escape.
POPULUS GRANDIDENTATA Michx. Large Toothed Aspen.
Common on hillsides, especially on newly cut-over land and open woods.
POPULUS TACAMAHACCA Mill. Balsam Poplar.
See Rhodora 21: 101. 1919.
Populus balsamifera Du Roi, not L.
Rare along shores of lake above Lower Dam in Upper Iowa River Valley.
POPULUS TREMULOIDES Michx. American Aspen. Quiver-leaf.
Common in habitats similar to those of *P. grandidentata*.
POPULUS BALSAMIFERA L. Cottonwood.
Populus deltoides Sargent of Gray's Manual and Britton and Brown.
Common along streams throughout region.
SALIX AMYGDALOIDES Anders. Peach-leaved Willow.
Common tree along rivers and similar locations.
SALIX BABYLONICA L. Weeping Willow.
Planted in lawns.
SALIX BEBBIANA Sarg. Bebb's Willow.
Salix rostrata Richards.
Small tree on edge of woods on south, shady hillsides and ravines or creeks.
SALIX CORDATA Muhl. Heart-leaved Willow.
Common bushy shrub or small tree along shores of rivers and creeks.
SALIX DISCOLOR Muhl.
Common prairie shrub, especially in lower lands.
SALIX HUMILIS Marsh.
Small shrub on upland prairies.
SALIX INTERIOR Rowlee. Sand-bar Willow.
Salix longifolia Muhl. not Lam.
Common willow of shrubby nature growing in colonies. This is one of the first trees to invade denuded lands along rivers.

Salix lucida Muhl. Shining Willow.

A single small tree found one mile north of Freeport in swamp and another two miles west of Ridgeway on low prairie land.

Salix nigra Marsh. Black Willow.

A common large tree in river valleys.

Salix petiolaris Smith.

Found on prairie swamp three miles north of Cresco in Howard County.

Salix tristis Ait.

Growing with *S. petiolaris.*

JUGLANDACEAE (Walnut Family)

Carya cordiformis (Wang.) K. Koch. Shell-bark Hickory.

A tree found chiefly in the valleys.

Carya ovata (Mill.) K. Koch. Shell-bark Hickory.

A common tree on north hillsides and upland woods. Commonly associated with *Quercus macrocarpa*, and the two together commonly dominate the north hillsides.

Juglans cinerea L. Butternut. White Walnut.

Frequent on hillsides, especially on protected hillsides, and on alluvial flood plains.

Juglans nigra L. Black Walnut.

Common in valleys in rich alluvial soils.

BETULACEAE (Birch Family)

Alnus incana (L.) Moench. Speckled or Hoary Alder.

A small tree found at Freeport and in Canoe Creek Valley growing in swamps and at springs. Rare.

Betula lutea Michx. f. Yellow Birch.

Found on protected hillside near McGregor.

Betula nigra L. River Birch.

Occasionally found in the Mississippi River Valley.

Betula papyrifera Marsh. Paper Birch.

Common on well drained hillsides, especially on the protected, south hillsides.

Carpinus caroliniana Walt. Blue Beech.

Common small tree, especially in linden-maple woods, where it occurs as undergrowth.

Corylus americana Walt. Hazel Nut.

Common shrub on prairies and open woods.

Corylus cornuta Marsh. Beaked Hazel Nut.

See Garden and Forest 8: 345.

Corylus rostrata Ait.

Rare in woods two miles west of Decorah.

Ostrya virginiana (Mill.) K. Koch. American Hop Hornbean.

In linden-maple woods as undergrowth. More common than *Carpinus caroliniana* with which it is often associated and to which it bears resemblance both in general appearance and environmental requirements.

FAGACEAE (Beech Family)

QUERCUS ALBA L. White Oak.
Common forest tree throughout the region, especially on the uplands and hillsides.

QUERCUS BICOLOR Willd. Swamp White Oak.
Rare tree found only in the Mississippi River Valley bordering sloughs and ponds.

QUERCUS BOREALIS Michx. f., var. MAXIMA (Marsh.) Ashe. Northern Great Oak.
See Rhodora 18: 45-52.
Common tree of uplands and hillsides.

QUERCUS ELLIPSOIDALIS J. E. Hill. Hill's Oak.
See Proc. Soc. Am. Foresters 11: 90. 1916.
Common tree of uplands and hillsides.

QUERCUS MACROCARPA Michx. Bur Oak.
Common tree on sunny hillsides and dry, well drained habitats. Commonly associated with *Carya ovata.*

QUERCUS MUHLENBERGII Engelm. Chestnut Oak. Chinquapin Oak.
Collected at Marquette on south, sunny hillside below calcareous cliff. Rare.

QUERCUS VELUTINA Lam. Black Oak.
Common large tree on uplands and hillsides throughout the region.

CASTANEA DENTATA (Marsh.) Borkh. American Chestnut.
Two large trees were found growing two miles north of Freeport near a farmhouse.

ULMACEAE (Elm Family)

CELTIS OCCIDENTALIS L. Hackberry.
Common large tree in valley woods.

ULMUS AMERICANA L. American Elm.
Very common dominant tree in valley woods. Commonly used as a shade tree.

ULMUS FULVA Michx. Red Elm. Slippery Elm.
Frequent in valleys with *Ulmus americana.*

ULMUS RACEMOSA Thomas. Cork or Rock Elm.
Ulmus Thomasi Sarg.
Not as common as the above two species; growing in similar habitats.

MORACEAE (Mulberry Family)

CANNABIS SATIVA L. Indian Hemp.
Common in alluvial pastures, fields. An introduced weedy plant.

HUMULUS LUPULUS L. Hop.
Common vine growing on edges of fields, roadsides, and thickets.

MORUS RUBRA L. Red Mulberry.
In open woods at Lansing and Freeport.

URTICACEAE (Nettle Family)

BOEHMERIA CYLINDRICA (L.) Sw. False Nettle.
Rare plant in moist, cool woods in Mississippi River Valley at Marquette.

LAPORTEA CANADENSIS (L.) Gaud. Wood or Canada Nettle.
Common weedy plant in valley woods in alluvial soils.
PARIETARIA PENNSYLVANICA Muhl. Pennsylvanian Pellitory.
Common weed in cool, moist woods.
PILEA PUMILA (L.) A. Gray. Clearweed.
Collected in valley woods in Hanover Township in Allamakee County and in swamp at Freeport.
URTICA PROCERA Muhl. Common Nettle.
See Rhodora 28: 192-195. 1926.
Urtica gracilis of Am. auth., not Ait.
Common weed in waste places, especially where ashes have been dumped or fires burned.

SANTALACEAE (Sandalwood Family)

COMANDRA UNBELLATA (L.) Nutt. Bastard Toad-flax.
Frequent in open woods and on prairies in large colonies.

ARISTOLOCHIACEAE (Birthwort Family)

ASARUM CANADENSE L. Wild Ginger.
Frequent in linden-maple woods throughout the region.

POLYGONACEAE (Buckwheat Family)

POLYGONELLA ARTICULATA Meissn. Joint Weed.
Abundant locally on open dune sands in Allamakee County northeast of Lycurgus and on sand terraces of the Upper Iowa River Valley.
POLYGONUM AVICULARE L. Knot Grass.
Common weedy plant in lawns, and along roads or paths.
POLYGONUM CONVOLVULUS L. Black Bindweed.
A weedy, climbing plant on fences and plants of the fields and gardens.
POLYGONUM COCCINEUM Muhl. Muhlenberg's Smartweed.
See Rh. 27: 162. 1925.
Collected in poorly drained pasture at Waukon.
POLYGONUM ERECTUM L. Weedy Knotweed.
In habitats similar to that of *P. aviculare*.
POLYGONUM HYDROPIPER L. Water Pepper.
I. S. C. Herbarium 71,643. Collected in Winneshiek County by Herbert Goddard, August 1, 1896.
POLYGONUM HYDROPIPEROIDES Michx. Mild Water Pepper.
Growing along edge of slough one mile east of New Albin. Growing in dense colonies, making a colorful show.
POLYGONUM LAPATHIFOLIUM L. Pale Persicaria.
Rhodora 23: 258-259.
Weedy plant along streams.
POLYGONUM PENNSYLVANICUM L. Pennsylvania Persicaria.
Common in fields, gardens, roadsides and along rivers.
POLYGONUM PERSICARIA L. Lady's Thumb.
Common in habitats similar to *P. pennsylvanica*.
POLYGONUM PUNCTATUM Ell. Smartweed.
Polygonum acre HBK.
Common in valley and prairie lowlands and swamps.

POLYGONUM SAGITTATUM L. Arrow-leaved Tear Weed.
In swamps four miles west of New Albin in Upper Iowa Valley.

POLYGONUM TENUE Michx. Slender Knotweed.
On sandy river terrace two miles east of Decorah.

POLYGONUM VIRGINIANUM L. Virginia Knotweed.
In moist, shady woods in Upper Iowa River Valley four miles west of New Albin.

POLYGONUM SCANDENS L. Climbing Buckwheat.
In thickets in Upper Iowa River Valley.

RUMEX ALTISSIMUS Wood. Great Tall Dock.
Common perennial weed in waste places throughout the region.

RUMEX BRITANNICA L. Great Water Dock.
In swamp at spring near Decorah.

RUMEX PATIENTIA L. Patience Dock.
In habitat similar to *Rumex Britannica.*

RUMEX CRISPUS L. Curled Dock.
In habitats similar to *Rumex altissimus.*

RUMEX VERTICILLATUS L. Swamp Dock.
Common on muddy banks of Mississippi River near New Albin and McGregor.

RUMEX ACETOSELLA L. Field, Wood, or Sheep Sorrel.
Common weed in fields and waste places.

CHENOPODIACEAE (Goosefoot Family)

CHENOPODIUM ALBUM L. Lamb's Quarters.
Common weed in disturbed lands, roadsides, gardens, etc.

CHENOPODIUM BOTRYS L. Jerusalem Oak, Feather Geranium.
On sandy river terrace in Hanover Township.

CHENOPODIUM GLAUCUM L. Oak-leaved Goosefoot.
Along roadside at McGregor.

CHENOPODIUM HYBRIDUM L. Hybrid Lamb's Quarters.
At Decorah in waste land.

ATRIPLEX PATULA L. Spear Scale.
A frequent weedy plant in gardens and disturbed lands.

SALSOLA KALI L. var. TENUIFOLIA G. F. W. Mey. Russian Thistle.
Salsola pestifer A. Nels.
On railroad tracks at Decorah.

AMARANTHACEAE (Amaranth Family)

AMARANTHUS BLITOIDES Wats. Spreading Pigweed.
In sandy, well drained soil.

AMARANTHUS GRAECIZANS L. Tumble Pigweed.
In sandy soil in corn fields in Hanover Township.

AMARANTHUS RETROFLEXUS L. Pigweed.
Common weed in gardens and fields.

FROELICHIA FLORIDANA (Nutt.) Moq.
Froelichia campestris Small.
On sandy river terrace four miles west of New Albin.

ACNIDA TUBERCULATA Moq. Water Hemp.
Common in disturbed alluvial soils along rivers.

ACNIDA TAMARISCINA (Nutt.) Wood.
In habitats similar to the above species of *Acnida.*

NYCTAGINACEAE (Four O'clock Family)

OXYBAPHUS NYCTAGINEUS (Michx.) Sweet. Wild Four-o'clock.
On prairies and sunny hillsides, especially in disturbed soil.

ILLECEBRACEAE (Knotwort Family)

ANYCHIA CANADENSIS (L.) BSP.
In open woods at Upper Dam. Rare.

AIZOACEAE

MOLLUGO VERTICILLATA L. Carpet Weed.
In sandy soil on river terrace and corn fields in Hanover Township in Allamakee County.

CARYOPHYLLACEAE (Pink Family)

ARENARIA LATERIFLORA L. Wood Sandwort.
In hazel thickets and open woods throughout region.
ARENARIA STRICTA Michx. Rock Sandwort.
Rare on limestone rocks near Highlandville.
CERASTIUM ARVENSE L.
A weedy plant growing throughout region.
CERASTIUM NUTANS Raf. Nodding Chickweed.
A weedy plant in open woods.
CERASTIUM VELUTINUM Raf. Barren Chickweed.
Collected on limestone talus below cliffs two miles southeast of Decorah and at Bluffton.
CERASTIUM VULGATUM L. Mouse-ear Chickweed.
Collected at Bluffton and Decorah on shady hillside in humus soil on calcareous rocks.
SAPONARIA OFFICINALIS L. Bouncing Bet. Soapwort.
A common escape from gardens.
SILENE DICHOTOMA L. Forked Catchfly.
On sandy soil along road at Upper Dam.
SILENE NIVEA DC. Snow Campion.
In swampy meadows.
SILENE ANTIRRHINA L. Sleepy Catchfly.
Common on rocky soil on sunny hillsides.
SILENE STELLATA (L.) Ait. f. Starry Campion.
On prairies near Ridgeway and Calmar.
SILENE NOCTIFLORA L. Night-flowering Catchfly.
Collected on roadside in Springfield Township.
STELLARIA MEDIA (L.) Cyrill. Chickweed.
Common in moist woods.
STELLARIA LONGIFOLIA Muhl.
Collected in ravine in woods near Upper Dam.

PORTULACACEAE (Purslane Family)

CLAYTONIA VIRGINICA L. Spring Beauty.
Common in alluvial soils in elm-ash woods.
PORTULACA OLERACEA L. Purslane.
Common annual weed in fields and gardens.

CERATOPHYLLACEAE (Hornwort Family)

Ceratophyllum demersum L. Hornwort.
In slough in Mississippi Valley near Lansing.

NYMPHAEACEAE (Water Lily Family)

Nelumbo lutea (Willd.) Pers. American Lotus.
See Rhodora 36: 23-24. 1934.
Nelumbo pentapetala (Walt.) Fernald.
Along shores of sloughs and lakes in Mississippi River Valley.
Nymphaea tuberosa Paine. White or Tuberous Water Lily.
See Rhodora 18: 120. 1916.
Castalia tuberosa (Paine) Greene.
In sloughs, slow flowing water and lakes in Mississippi River Valley.
Nuphar advena Ait. Spatter Dock, Yellow Pond Lily or Cow Lily.
See Rhodora 21: 186. 1919; 39: 333. 1937.
Nymphozanthus advena (Ait.) Fernald.
Common in sloughs and habitats similar to the above species.
Brasenia Schreberi Gmel.
Collected in pond in Upper Iowa River Valley west of New Albin.

RANUNCULACEAE (Buttercup Family)

Actaea alba (L.) Mill. White Baneberry.
In linden-maple woods.
Actaea rubra (Ait.) Willd. Red Baneberry.
In habitats similar to the above species.
Anemone patens L. var. Wolfgangiana (Bess.) Koch.
On sunny, rocky hillsides in Upper Iowa and Mississippi River Valleys. Not common.
Anemone canadensis L. White Anemone.
Common on prairies and open woods.
Anemone cylindrica Gray. Thimbleweed.
On rocky hillsides and prairies.
Anemone quinquefolia L. Wood Anemone.
Common in woodlands and upland prairies.
Anemone virginiana L. Virginia Anemone.
Frequent in open woods.
Anemonella thalictroides (L.) Spach. Rue Anemone.
In upland oak woods. Common locally.
Aquilegia canadensis L. Wild Columbine.
Common on rocks and rocky soils in shade or full sun.
Caltha palustris L. Marsh Marigold.
In swamps and spring horizons. Becoming rare.
Clematis verticillaris DC. Whorled Clematis.
On limestone cliff at Decorah.
Clematis virginiana L. Virgin's Bower.
Common in thickets and brush in river valleys.
Delphinium virescens Nutt. White Delphinium.
Delphinium Penardi of Rob. & Fern.
I. S. C. Herbarium 71,689. Collected by Herbert Goddard in Winneshiek County, June 25, 1895.

HEPATICA ACUTILOBA DC. Liver Leaf. Hepatica.
Common vernal herb in linden-maple woods. Colors range from white to blue and purple.

HYDRASTIS CANADENSIS L. Golden Seal.
In woods at Lower Dam. Rare.

RANUNCULUS TRICHOPHYLLUS Chaix. White Water Crowfoot.
See Rhodora 38: 26. 1936.
R. aquatilis L. var. *capillaceus* DC.
In cold waters at spring near the mouth of Canoe Creek.

RANUNCULUS ABORTIVUS L. Small-flowered Crowfoot.
In valley woods on alluvial soils and in moist upland woods.

RANUNCULUS FASCICULARIS Muhl. Prairie Buttercup.
On sunny, well drained hillsides.

RANUNCULUS HISPIDUS Michx. Hairy Swamp Buttercup.
In swamps one mile south of Lansing. Rare.

RANUNCULUS RECURVATUS Poir. Hooked Crowfoot.
Frequent in moist woods in humus soil.

RANUNCULUS RHOMBOIDEUS Goldie. Rhombic-leaved Crowfoot.
On sunny, well drained hillside two miles southeast of Decorah.

RANUNCULUS SCELERATUS L. Cursed Crowfoot.
Along muddy shores of Mississippi River and above Lower Dam on river mud flats.

RANUNCULUS SEPTENTRIONALIS Poir. Woodland Buttercup.
In moist, alluvial soils in elm-ash woods of valleys.

RANUNCULUS PENNSYLVANICUS L. f.
In swamps in Mississippi Valley.

THALICTRUM DASYCARPUM Fish. & Lall. Tall Meadow Rue.
In low prairies and swamps.

THALICTRUM DIOICUM L. Spring Meadow Rue.
Common in linden-maple woods.

MENISPERMACEAE (Moonseed Family)

MENISPERMUM CANADENSE L. Moonseed.
A vine found commonly in thickets.

BERBERIDACEAE (Barberry Family)

CAULOPHYLLUM THALICTROIDES (L.) Michx. Blue Cohosh.
Common herb in linden-maple woods.

PODOPHYLLUM PELTATUM L. May Apple, Mandrake.
Common in open woods and pastures in the valleys throughout the region.

BERBERIS VULGARIS L. European Barberry.
Collected in open woods and pastures south of Decorah.

BERBERIS THUNBERGII DC. Japanese Barberry.
Commonly cultivated in yards.

PAPAVERACEAE (Poppy Family)

SANGUINARIA CANADENSIS L. Bloodroot.
Common in linden-maple woods, but becoming scarce because of picking and other sources of destruction.

CHELIDONIUM MAJUS L. Celandine.

Becoming established in the linden-maple woods in Pleasant Township in Winneshiek County. Frequently crowds other native herbs and dominates entire areas.

FUMARIACEAE (Fumitory Family)

CORYDALIS AUREA Willd.

Collected at Decorah on rocky, sunny hillside.

DICENTRA CUCULLARIA (L.) Bernh.

Common in linden-maple woods; blooms in spring before leaves appear on trees and dies during the first of June.

DICENTRA CANADENSIS (Goldie) Walp. Squirrel's Corn.

Occurring in same habitat as *Dicentra Cucullaria,* but not as common. Found at Decorah and Canoe Creek.

CRUCIFERAE (Mustard Family)

ARABIS DENTATA T. & G. Toothed Arabis.

In woods at Pallisade Park in Decorah.

ARABIS CANADENSIS L. Sickle-pod.

In upland woods at Bluffton and Decorah.

ARABIS HIRSUTA (L.) Scop. Hairy Rock Cress.

In open woods on south hillside at Decorah.

ARABIS LYRATA L. Rock Cress.

Common on limestone cliffs and rocky soils.

ARABIS LAEVIGATA (Muhl.) Poir. Smooth Rock Cress.

On rocky, sunny hillside at Decorah. Not common.

CAMELINA SATIVA Crantz. False Flax.

I. S. C. Herbarium 41,790. Collected by Holway, June 22, 1893, at Decorah.

BERTEROA INCANA (L.) DC. Hoary Alyssum.

Frequent on roadsides and waste places.

BRASSICA JUNCEA (L.) Casson. Indian Mustard.

Common weed in fields.

BRASSICA ARVENSIS (L.) Ktze. Field Mustard.

Common weedy plant in grain fields.

BRASSICA NIGRA (L.) Koch. Black Mustard.

Common weed in fields.

CARDAMINE BULBOSA (Schreb.) BSP. Bulbous Cress.

Common plant in swamps and at springs.

CARDAMINE PENNSYLVANICA Muhl. Pennsylvania Bitter Cress.

Along muddy shores of a Mississippi slough near Lansing.

CAPSELLA BURSA-PASTORIS (L.) Medic. Shepherd's Purse.

Common in lawns, fields, and similar habitats.

DENTARIA LACINIATA Muhl. Toothwort.

Frequent in linden-maple woods.

DRABA REPTANS (Lam.) Fernald.

See Rhodora 36: 368. 1934.

Draba caroliniana Walt.

On dry, sunny hillsides, on calcareous soil and rocks.

ERYSIMUM CHEIRANTHOIDES L. Worm-seed Mustard.
See Rydberg's Flora of the Plains and Prairies.
Cheirinia cheiranthoides (L.) Link.
In linden-maple woods on talus below cliff in humus soil.

ERYSIMUM PARVIFLORUM Nutt. Small-flowered Prairie Rocket.
Cheirinia inconspicua (S. Wats.) Rydb.
In gravel soil along railroad near McGregor.

LEPIDIUM DRABA L. Hoary Cress.
Along roadside at Calmar. Rare.

LEPIDIUM DENSIFLORUM Schrad.
Lepidium apetalum of auth. not Willd.
A weedy plant in gardens, lawns, and waste places.

LEPIDIUM VIRGINICUM L. Virginia Pepper Grass.
A common weedy plant growing with *L. densiflorum*.

RORIPA ARMORACIA (L.) Hitchc. Horse Radish.
Radicula Armoracia (L.) Robinson.
An escape from cultivation. Frequent in fields and gardens.

RORIPA NASTURTIUM-AQUATICUM (L.) Schinz & Thell. True Water Cress.
Radicula Nasturtium-aquaticum (L.) Britton and Rendle.
Common in swift moving, cold water streams from springs in the vicinity of Decorah.

RORIPA HISPIDA var. GLAERATA Lundell. Marsh Water Cress.
See Rhodora 30: 133. 1928.
Radicula palustris of Am. auth. not Moench.
In Mississippi Valley along muddy shores of sloughs, and at Lower Dam in alluvial fields.

RORIPA SESSILIFLORA (Nutt.) Hitchc. Sessile-flowered Cress.
Radicula sessiliflora (Nutt.) Greene.
On muddy shores of slough in Mississippi Valley north of Lansing.

RORIPA SYLVESTRIS (L.) Bess. Yellow Cress.
Radicula sylvestris (L.) Druce.
Frequent in moist ravine and along shores and banks of rivers.

SISYMBRIUM ALTISSIMUM L. Tumble Mustard.
In sandy, well drained soil along railroad track at Calmar and New Albin.

SISYMBRIUM CANESCENS Nutt. Canescent Hedge Mustard.
In fields and sunny hillsides at Decorah.

SISYMBRIUM OFFICINALE (L.) Scop. Hedge Mustard.
In fields and around buildings at Decorah.

THLASPI ARVENSE L. Penny Cress.
Along railroad track one mile south of Lansing.

CAPPARIDACEAE (Caper Family)

POLANISIA GRAVEOLENS Raf. Clammy Weed.
In gravel pit at Union City and Calmar. Not common.

CRASSULACEAE (Orpine Family)

PENTHORUM SEDOIDES L. Ditch Stonecrop.
Along streams and moist places.

SEMPERVIVUM TECTORUM L. House Leek.
An escape from cultivation, especially common in old graveyards.

SAXIFRAGACEAE (Saxifrage Family)

CHRYSOSPLENIUM IOWENSE Rydb. Iowa Golden Saxifrage.
Rare in open, swampy area on south hillside in open woods near Decorah. Associated with the tree moss, *Climacium americanum* on calcareous talus rocks.

HEUCHERA RICHARDSONII R. Br. Alum Root.
See Rhodora 35: 111. 1933.
In crevices in limestone cliffs and on sandy river terraces and open woods on sunny hillside at Upper Dam.

MITELLA DIPHYLLA L. Mitre-wort. Bishop's Cap.
Common in linden-maple woods throughout the region.

RIBES AMERICANUM Mill. Black Wild Currant.
Ribes floridum L'Her.
Frequent in valley woods throughout the area and collected on prairie at Calmar.

RIBES CYNOSBATI L. Prickly Gooseberry.
Common on limestone cliffs and rocks in woodlands.

RIBES MISSOURIENSE Nutt. Missouri Gooseberry.
Ribes gracile Michx.
Common in valley woods and hillsides.

RIBES HIRTELLA (Michx.) Spach.
A few bushes in open valley woods near Decorah.

SAXIFRAGA PENNSYLVANICA L. Swamp Saxifrage.
Collected on prairie swamp a few miles west of Ridgeway and on south hillside in open woods, where the humus soil was mixed with limestone talus.

SULLIVANTIA RENIFOLIA Rosendahl. Sullivant's Saxifrage.
See Univ. of Minn. Studies. Biol. Sci. 6: 40, pl. 34. 1927.
Sullivantia Sullivantii (T. & G.) Britton.
In crevices in limestone cliffs on south shady hillside at Highlandville and Lansing.

HAMAMELIDACEAE (Witch-hazel Family)

HAMAMELIS VIRGINIANA L. Witch-hazel.
On south, shady hillsides in linden-maple woods in Mississippi Valley near Lansing and at McGregor.

PLATANACEAE

PLATANUS OCCIDENTALIS L. Sycamore.
I. S. C. Herbarium 140,981. Collected at McGregor by Pammel, May 30, 1918.

ROSACEAE (Rose Family)

AGRIMONIA GRYPOSEPALA Wallr. Tall Hairy Agrimony.
Frequent in upland oak-hickory woods.

AMELANCHIER CANADENSIS (L.) Medic. Canada Service Berry.
Common shrub or small tree in woods throughout area.

AMELANCHIER HUMILIS Wiegand. Low Service Berry.
See Rhodora 14: 126-141. 1912.
In sandy soils at Upper Dam and on limestone cliffs throughout the region.

AMELANCHIER LAEVIS Wiegand. Smooth Juneberry.
Collected at Decorah in open woods.

CRATAEGUS CRUS-GALLI L. Cockspur Thorn.
Collected at McGregor on sunny hillside and in open woods.

CRATAEGUS CALPODENDRON (Ehrh.) Medic.
C. tomentosa of auth. not L.
Frequent in woods in the Upper Iowa River Valley.

CRATAEGUS MOLLIS (T. & G.) Scheel. Woolly Thorn.
On sunny hillside near Decorah and in open valley at Highlandville.

CRATAEGUS MOLLIS (T. & G.) Scheele. Wooly Thorn.
Collected in Mississippi Valley a few miles north of Lansing.

CRATAEGUS PUNCTATA Hook. Dotted Thorn.
Common in valleys throughout the region.

FRAGARIA VESCA L. var. AMERICANA Porter. Wood Strawberry.
Fragaria americana (Porter) Britton.
Common in woods throughout region.

FRAGARIA VIRGINIANA Duchesne. Virginia Strawberry.
Common in open woods and prairies.

GEUM CANADENSE Jacq. White Avens.
Common on prairies and in open woods.

GEUM STRICTUM Ait. Yellow Avens.
In swampy land on prairie near Ossian.

GEUM TRIFLORUM Pursh. Prairie Smoke or Prairie Avens.
Sieversia triflora (Pursh) R. Br.
Rare on sandy river terrace near Freeport and on well drained prairie land one mile east of Ridgeway.

PHYSOCARPUS OPULIFOLIUS (L.) Maxim., var. INTERMEDIUS (Rydb.) Robinson. Ninebark.
Common on well drained hillsides and cliffs.

POTENTILLA ARGENTEA L. Silvery Cinquefoil.
In open woods near McGregor.

POTENTILLA ARGUTA Pursh. Silvery Cinquefoil.
On sandy soil of river terraces and on well drained prairie lands.

POTENTILLA FRUTICOSA L. Shrubby Cinquefoil.
Dasiphora fruticosa (L.) Rydb.
On south, sunny and exposed cliff at Bluffton.

POTENTILLA NORVEGICA L. var. HIRSUTA (Michx.) Lehm. Rough Cinquefoil.
See Rhodora 28: 213-214. 1926.
Potentilla monspeliensis L.
Common weedy plant in waste lands.

POTENTILLA SIMPLEX Michx. Cinquefoil.
See Rhodora 33: 188. 1931.
Potentilla canadensis of Am. auth. not L.
Common on prairies and in open woods.

POTENTILLA TRIDENTATA Ait. Three-toothed Cinquefoil.
I. S. C. Herbarium 12,622. Collected by A. S. Hitchcock near Hesper.

PRUNUS AMERICANA Marsh. Wild Plum.
Common plum growing in thickets throughout the region.

PRUNUS NIGRA Ait. Black Plum.
Not common in open woods at Decorah and Canoe Creek.
PRUNUS PENNSYLVANICA L. f. Pin Cherry.
Frequent in upland oak woods, and in disturbed lands. Pioneer woody species in denuded areas.
PRUNUS PUMILA L. Sand Cherry.
On sandy soil below sandstone cliff in Mississippi Valley.
PRUNUS SEROTINA Ehrh. Tall Choke Cherry.
Common in upland oak woods.
PRUNUS VIRGINIANA L. Small Choke Cherry.
Common in thickets along edges of woods, in open woods, and on prairies along edges of fields.
MALUS IOENSIS (Wood.) Bailey. Wild Crab Apple.
Pyrus ioensis (Wood.) Britton.
Common in open woods and thickets.
ROSA ARKANSANA Porter. Prairie Rose.
Common on prairies, sunny hillsides, and open woods throughout the region.
ROSA BLANDA Ait. Meadow Rose.
Frequent in the wooded sections.
ROSA CAROLINA L. Carolina Rose.
See Rhodora 20: 19. 1918.
Collected one mile north of Freeport on north, sunny hillside.
RUBUS ALLEGHENIENSIS Porter. Blackberry.
In open woods in Hanover Township in Upper Iowa River Valley.
RUBUS NEGLECTUS Peck.
Rubus occidentalis x *Rubus strigosus.*
In open woods at Lower Dam in Upper Iowa River Valley.
RUBUS OCCIDENTALIS L. Black Raspberry. Black-caps.
Common in habitats similar to *Rubus strigosus.*
RUBUS PUBESCENS Raf. Dwarf Red Raspberry.
Rubus triflorus Richards; *Rubus americanus* (Pers.) Britt.
In moist linden-maple woods on south, shady hillsides near Highlandville and in prairie swamp three miles north of Cresco.
RUBUS STRIGOSUS Michx. American Red Raspberry.
Common in thickets and open woods throughout region.
SPIRAEA ALBA Du Roi. Prairie Spiraea.
Spiraea salicifolia of Am. auth., not L.
Collected on prairie near Ridgeway.
SORBUS AUCUPARIA (L.) Ehrh. Mountain Ash.
Escape on edge of oak-hickory woods at Conover.

LEGUMINOSAE (Pea Family)

AMORPHA CANESCENS Pursh. Lead Plant. Shoe String.
Common low shrub on prairies and sunny hillsides.
AMORPHA FRUTICOSA L. False Indigo.
Frequent in valleys on edge of rivers and swamps.
AMPHICARPA BRACTEATA (L.) Fernald. Hog Peanut.
See Rhodora 35: 276. 1933.
Amphicarpa monoica (L.) Ell.
Frequent in open woods throughout the region.

APIOS AMERICANA Medic. Groundnut.
See Rhodora 36: 88-89. 1934.
Apios tuberosa Moench.
Collected at Freeport in swamp and on prairie near Calmar.

ASTRAGALUS CANADENSIS L. Canada Milk Vetch.
Collected on prairie near Calmar.

ASTRAGALUS CARYOCARPUS Ker. Ground Plum.
Collected at Decorah on sunny, rocky, well drained hillside.

BAPTISIA LEUCANTHA T. & G. White Wild Indigo.
Common herb on prairie along Milwaukee Railroad from Calmar to Ridgeway.

BAPTISIA LEUCOPHAEA Nutt. Yellow Wild Indigo.
B. bracteata of auth., not of (Muhl.) Ell.
Frequent on prairie along Milwaukee Railroad from Calmar to Ridgeway. Not as common as *Baptisia leucantha*.

CARAGANA ARBORESCENS Lam. Shrubby Pea Tree.
Planted in lawn at Decorah.

CASSIA FASCICULATA Michx. Partridge Pea.
C. chamaecrista of auth., not L.
Found at Hanover in Allamakee County in Upper Iowa River Valley on sandy soils and in gravel pits.

CROTALARIA SAGITTALIS L. Rattle-box Weed.
Collected only near New Albin along Milwaukee Railroad in gravel along railroad.

DESMODIUM ACUMINATUM Michx. Pointed-leaved Tick-trefoil.
Desmodium grandiflorum (Walt.) DC.
Growing in upland red and white oak woods.

DESMODIUM CANADENSE (L.) DC. Canadian Tick-trefoil.
Common on prairies and in open woods.

DESMODIUM ILLINOENSE Gray. Illinois Tick-trefoil.
In Hanover Township on sandy river terrace.

GLEDITSIA TRIACANTHOS L. Honey Locust.
At Fort Atkinson a single tree was found on flood plain and also at Marquette in Mississippi Valley.

GYMNOCLADUS DIOICA (L.) Koch. Kentucky Coffee Tree.
Rare in Mississippi River Valley at Lansing.

LATHYRUS OCHROLEUCUS Hook. White Sweet Pea.
Rare in upland open red oak woods.

LATHYRUS PALUSTRIS L. Swamp Sweet Pea.
Rare in swamp two miles south of Lansing in Mississippi River Valley.

LATHYRUS VENOSUS Muhl. Prairie Sweet Pea.
Common on prairies and open woods throughout region.

LESPEDEZA CAPITATA Michx. Round-headed Bush Clover.
Common on prairies and sunny hillsides.

LESPEDEZA LEPTOSTACHYA Engelm.
Collected on prairie near Ridgeway.

LUPINUS PERENNIS L. Perennial Lupine.
Collected by Holway at Decorah, June 4, 1893. I. S. C. Herbarium 28,811.

MEDICAGO LUPULINA L. Black or Hop Medic.
A weedy plant along roadsides and waste places.

MEDICAGO SATIVA L. Alfalfa.
A common escape from cultivation.

MELILOTUS ALBA Desv. White Sweet Clover.
A common escape from cultivation, establishing itself everywhere, crowding out the native vegetation.

MELILOTUS OFFICINALIS (L.) Lam. Yellow Sweet Clover.
With characteristics of *M. alba* but not as common.

PETALOSTEMON CANDIDUS (Willd.) Kuntz. White Prairie Clover.
Common on prairies and sunny hillsides.

PETALOSTEMON PURPUREUM (Vent.) Rydb. Purple Prairie Clover.
Common on prairies.

PSORALEA ARGOPHYLLA Pursh. Silver-leaf Psoralea.
Rare on sandy river terrace in Hanover Township.

PSORALEA ESCULENTA Pursh. Prairie Apple or Pomme de Terre.
Rare on sunny hillside at Decorah.

ROBINIA PSEUDO-ACACIA L. Black Locust.
Planted in lawns at Decorah.

STROPHOSTYLES HELVOLA (L.) Britton. Trailing Wild Bean.
In fields of sandy soil four miles south of Lansing in Mississippi River Valley.

TEPHROSIA VIRGINIANA (L.) Pers. Hoary Pea.
Rare on sandy river terraces in Upper Iowa River Valley ten miles west of New Albin.

TRIFOLIUM HYBRIDUM L. Hybrid Clover, Alsike Clover.
Common throughout the region. Introduced from Europe.

TRIFOLIUM PRATENSE L. Red Clover.
Common escape from cultivation.

TRIFOLIUM PROCUMBENS L. Low Hop-Clover.
A weedy plant in disturbed land.

TRIFOLIUM REPENS L. White Clover.
On habitat similar to the above species.

VICIA AMERICANA Muhl. American Vetch.
Common on upland prairies and along roads.

VICIA SATIVA L.
Collected at Calmar on prairie along Milwaukee Railroad.

VICIA VILLOSA L. Hairy Vetch.
Collected in Pleasant Township along roadside.

LINACEAE (Flax Family)

LINUM SULCATUM Riddell. Wild Yellow Flax.
On sunny hillsides and prairie uplands.

LINUM USITATISSIMUM L. Domestic Flax.
An occasional escape from cultivation.

OXALIDACEAE (Oxalis Family)

OXALIS STRICTA L. Yellow Wood-sorrel.
Common weedy plant throughout region.

OXALIS VIOLACEA L. Violet Wood-sorrel.
Common on sunny, rocky hillsides and prairies.

GERANIACEAE (Geranium Family)

GERANIUM MACULATUM L. Wild Geranium.
Common in upland oak woods.

RUTACEAE (Rue Family)

ZANTHOXYLUM AMERICANUM Mill. Prickly Ash.
Common shrub in open woods in river valleys. Generally grows in large colonies.

POLYGALACEAE (Milkwort Family)

POLYGALA SANGUINEA L. Purple Milkwort.
Common on prairie especially where soil has been disturbed.

POLYGALA SENEGA L. Seneca Snakeroot.
Common in open woods and on upland prairie.

POLYGALA VERTICILLATA L. Whorled Milkwort.
Collected one mile east of Ridgeway on lowland prairie.

EUPHORBIACEAE (Spurge Family)

ACALYPHA VIRGINICA L. Virginia Three-seeded Mercury.
Common in shady woods and swamps.

EUPHORBIA COMMUTATA (Engelm.) Kl. & Garcke. Tinted Spurge.
Collected on sandy bank along river at Upper Dam.

EUPHORBIA COROLLATA L. Flowering Spurge.
Common on upland prairies, sunny hillsides, open woods, and even an early perennial invader in denuded lands.

EUPHORBIA CYPARISSIAS L. Cypress Spurge.
An escape near graveyards. European.

EUPHORBIA ESULA L. Leafy Spurge.
See Rh. 39:49-50. 1937. Certain Botanists claim that *E. virgata* Waldst. & Kit., but not *E. Esula* occurs in America. However, the two have not been shown to be distinct.
Collected at Canoe Creek Church. Rare in this region.

EUPHORBIA HETEROPHYLLA L. Various-leaved Spurge.
In sandy soils and open places at Upper Dam and in valley of the Upper Iowa River in Hanover Township.

EUPHORBIA GLYPTOSPERMA Engelm. Ridge-seeded Spurge.
A weedy plant growing in sandy soil.

EUPHORBIA MACULATA L. Spotted Spurge.
A weedy plant growing in fields and waste places.

EUPHORBIA MARGINATA Pursh. Snow-on-the-Mountain.
An escape from gardens; a southern species.

EUPHORBIA PRESLII Guss. Upright Spotted Spurge.
Common weedy plant in open places, fields, gardens, and railroad tracks.

ANACARDIACEAE (Sumac Family)

RHUS TYPHINA L. Staghorn Sumac.
Rhus hirta (L.) Sudworth.
On Edge of woods on south hillside in Mississippi Valley.

RHUS GLABRA L. Smooth Upland Sumac.

Common shrub on prairies, open, sunny hillsides, and woods. Common pioneer of woody vegetation.

RHUS TOXICODENDRON L. Poison Ivy.

Common on upland prairies and thickets.

CELASTRACEAE (Staff Tree Family)

CELASTRUS SCANDENS L. Bitter Sweet.

In open woods and thickets, limestone cliffs, and prairies.

EVONYMUS ATROPURPUREUS Jacq. Wahoo.

In moist woods in valleys.

STAPHYLEACEAE (Bladder Nut Family)

STAPHYLEA TRIFOLIA L. American Bladdernut.

Common in moist, thick linden-maple woods, especially on south hillsides.

ACERACEAE (Maple Family)

ACER NEGUNDO L.

Common in open woods, and along edges of fields.

ACER PLATANOIDES L. Norway Maple.

Occasionally planted in lawns.

ACER SACCHARINUM L. Soft Sugar Maple.

Common in river valleys, especially along river banks. Commonly planted as a shade tree.

ACER SACCHARUM Marsh. Sugar Maple.

Common tree, especially on the south hillsides, in the Mississippi Valley. Not as common as the variety.

ACER SACCHARUM Marsh. var. NIGRUM (Michx. f.) Britton. Black Sugar Maple.

A dominant tree in the climax forest of the region.

ACER SPICATUM Lam. Mountain Maple.

A rare tree in linden-maple woods along west hillsides of Mississippi River Valley at Lansing.

SAPINDACEAE (Soapberry Family)

AESCULUS GLABRA Willd. Ohio Buckeye.

Planted in lawns in Decorah.

AESCULUS HIPPOCASTANUM L. Horse Chestnut.

Planted in lawns at Decorah.

BALSAMINACEAE (Touch-me-not Family)

IMPATIENS BIFLORA Walt. Orange Touch-me-not.

Common in swamp land at spring horizon and in moist, shady woods.

IMPATIENS PALLIDA Nutt. Yellow Touch-me-not.

In habitats similar to the above species.

RHAMNACEAE (Buckthorn Family)

RHAMNUS ALNIFOLIA L'Her. Alder-leaved Buckthorn.
Occasionally found on moist, south hillsides on calcareous talus in open woods along Dugway road at Decorah, and three miles west of Decorah in Upper Iowa River Valley.

CEANOTHUS AMERICANUS L. New Jersey Tea.
Common on prairies and open woods throughout the region.

CEANOTHUS OVATUS Desf. var. PUBESCENS T. & G. Small Red-root.
Rare on sunny hillsides in Hanover Township and four miles north of Lansing on Mississippi Valley hills.

VITACEAE (Vine Family)

PARTHENOCISSUS QUINQUEFOLIA (L.) Planch. Virginia Creeper.
Psedera quinquefolia (L.) Greene.
Common on buildings, fences, and in open woods.

VITIS VULPINA L. Wolf Grape.
Common in thickets in valleys throughout the region.

VITIS BICOLOR Le Conte. Winter Grape.
Rare on hills in the vicinity of McGregor.

TILIACEAE (Linden Family)

TILIA GLABRA Vent. Basswood, Linden.
See Bot. Gaz. 66: 424. 1918. Bul. Torr. Bot. Club 54: 235. 1927.
Tilia americana L. in part.
A climax species associated with black maple on protected hillsides.

MALVACEAE (Mallow Family)

ABUTILON THEOPHRASTI Medic. Velvet Leaf.
Common weed in valley corn fields.

ALTHAEA ROSEA Cav. Hollyhock.
An escape from cultivation.

CALLIRRHÖE TRIANGULATA (Leavenw.) Gray. Poppy-mallow.
Collected on sandy river terraces in Upper Iowa River Valley four miles west of New Albin.

HIBISCUS MILITARIS Cav. Rose Mallow.
Rare plant in swampy lands in Mississippi Valley.

HIBISCUS TRIONUM L. Venice Mallow. Flower-of-the-Hour.
A weedy plant on sandy soils at Decorah.

MALVA VERTICILLATA L. Whorled Mallow.
A weedy plant in barnyards, gardens, etc. Rare.

MALVA ROTUNDIFOLIA L. Cheeses.
A weedy plant common in barn yards, gardens, and roadsides.

NAPAEA DIOICA L. Glade Mallow.
Frequent plant in open, weedy pastures in Upper Iowa River Valley at Decorah and Bluffton.

HYPERICACEAE (St. John's-wort Family)

HYPERICUM ASCYRON L. St. John's-wort.
Rare plant on open prairie.

HYPERICUM CANADENSE L.
On moist, low land of prairie in Winneshiek County.

HYPERICUM MUTILUM L. Slender St. John's-wort.
At McGregor in moist, swampy locations and on lowland prairie east of Ridgeway.

HYPERICUM PERFORATUM L. Common St. John's-wort.
A weedy plant collected on prairie several miles west of Waukon along highway.

HYPERICUM PUNCTATUM Lam. Spotted St. John's-wort.
In open woods throughout the region.

CISTACEAE (Rockrose Family)

HELIANTHEMUM VILLOSA Ell. Pinweed.
See Rhodora 21: 36. 1919.
Helianthemum majus Michx.
On upland prairies and steep hillsides.

LECHEA TENUIFOLIA Michx. Pinweed.
On sandy soil of river terrace in Hanover Township.

VIOLACEAE (Violet Family)

VIOLA CANADENSIS L. Canadian Violet.
In woods on south hillside along Dugway road at Decorah.

VIOLA PAPILIONACEA Pursh. Hooded Blue Violet.
In woods throughout area.

VIOLA ERIOCARPA Schw. Yellow Violet.
See Rhodora 23: 275. 1922.
Viola scabriuscula Schwein.
Common in woods throughout the region.

VIOLA PEDATA L. Bird-foot or Crow-foot Violet.
Collected on prairie near Calmar and on sandy river terrace near Upper Dam in Upper Iowa Valley and in Mississippi Valley north of Lansing on sandy soil.

VIOLA PEDATIFIDA Don. Prairie Violet.
Common on upland prairies.

VIOLA SORORIA Willd.
Common in woods throughout the region.

CACTACEAE (Cactus Family)

OPUNTIA RAFINESQII Engelm. Prickly Pear Cactus.
Collected by Ada Hayden on sandy talus below cliff six miles northeast of Elon along Village Creek in Allamakee County.

THYMELAEACEAE (Mezereum Family)

DIRCA PALUSTRIS L. Leatherwood.
A small shrub in linden-maple woods in Canoe Creek Valley and two miles south of Lansing.

ELAEAGNACEAE (Oleaster Family)

ELAEAGNUS ANGUSTIFOLIA L. Russian Olive.
Planted in lawns at Decorah.

LYTHRACEAE (Loosestrife Family)

LYTHRUM ALATUM Pursh. Winged Loosestrife.
Common in swamps and at springs throughout the region.

ONAGRACEAE (Evening Primrose Family)

CIRCAEA ALPINA L. Small Enchanter's Nightshade.
Rare in moist, cool, linden-maple woods on south hillside beneath high cliff.

CIRCAEA LATIFOLIA Hill. Enchanter's Nightshade.
See Rhodora 19:87. 1917.
Circaea lutetiana of Am. auth., not L.
Common in upland oak woods.

EPILOBIUM ANGUSTIFOLIUM L. Narrow-leaved Willow-herb.
On recently disturbed soil along road near Decorah and Conover.

EPILOBIUM COLORATUM Muhl. Purple-leaved Willow-herb.
I. S. C. Herbarium 71,927. Collected in Winneshiek County by Herbert Goddard, July 6, 1895.

EPILOBIUM ADENOCAULON Haussk. Northern Willow-herb.
I. S. C. Herbarium 15,010. Collected in Winneshiek County along Yellow River, July 27, 1924, by L. H. Pammel.

LUDWIGIA POLYCARPA Short and Peter. Many Fruited Ludwigia.
On edge of pond in Mississippi Valley.

OENOTHERA BIENNIS L. Evening Primrose.
On upland prairies and waste lands.

OENOTHERA SERRULATA Nutt. Tooth-leaved Primrose.
Common on sunny hillsides and well drained prairies.

OENOTHERA PERENNIS L. Small Sundrops.
See Bull. Torr. Bot. Club, 64:287-304. 1937.
O. pumila L.
In low prairie land one mile east of Ridgeway.

OENOTHERA RHOMBIPETALA Nutt. Rhombic-petalled Primrose.
On sandy river terraces near Freeport and in Hanover Township in old, idle fields.

ARALIACEAE (Ginseng Family)

ARALIA RACEMOSA L. American Spikenard.
Frequent in linden-maple woods, especially in deep ravines.

ARALIA NUDICAULIS L. Virginian Sarsaparilla.
Common in upland oak woods.

PANAX QUINQUEFOLIUM L. Ginseng.
Formerly common in very shady linden-maple woods, but now rare because of extensive collecting of its roots, which are of medical value.

UMBELLIFERAE (Parsley Family)

ANGELICA ATROPURPUREA L. Great Purple-stemmed Angelica.
Not common in alluvial soils along rivers.

CARUM CARVI L. Caraway.
A common escape from cultivation.

CICUTA MACULATA L. Water Hemlock.
In swamp on prairies near Calmar and Ridgeway.

CONIUM MACULATUM L. Poison Hemlock.
In alluvial soil at Decorah.
CRYPTOTAENIA CANADENSIS (L.) DC. Honewort.
Common in moist woods.
DAUCUS CAROTA L. Carrot.
A weed entering prairie along roadways in Allamakee County.
ERYNGIUM YUCCIFOLIUM Michx. Button Snakeroot.
Common on upland prairies at Calmar and Ridgeway.
HERACLEUM LANATUM Michx. Cow-parsnip.
Occasionally found in low open lands on alluvial soils.
OSMORRHIZA CLAYTONI (Michx.) Clarke. Woolly Sweet Cicely.
Frequent in woods throughout the area.
OSMORRHIZA LONGISTYLIS (Torr.) DC. Anise-root.
In habitats similar to the above species.
PSEUDOTAENIDIA NUTTALLII DC. False Taenidia.
Rare on prairie land in Madison Township.
PASTINACA SATIVA L. Parsnip.
A weedy plant frequenting roadsides and waste places.
SANICULA CANADENSIS L. Short-styled Snakeroot.
Common in upland oak woods.
SANICULA GREGARIA Bicknell. Clustered Snakeroot.
Common in woods throughout the region.
SANICULA MARILANDICA L. Black Snakeroot.
Common in woods throughout the region.
SIUM CICUTAEFOLIUM Schrank. Hemlock Water-Parsnip.
In swamps in Mississippi Valley at Marquette.
TAENIDIA INTEGERRIMA (L.) Drude. Yellow Pimpernell.
Frequent on prairies and open woods.
THASPIUM BARBINODE (Michx.) Nutt. Hairy Jointed Meadow Parsnip.
Collected at McGregor in open oak-hickory woods.
ZIZIA AUREA (L.) Koch. Golden Meadow-Parsnip.
Common on prairies and open woods throughout the region.
ZIZIA CORDATA (Walt.) DC. Heart-leaved Alexander.
On prairies at Calmar and Ridgeway. Not as common as the above species.

CORNACEAE (Dogwood Family)

CORNUS ALTERNIFOLIA L. Alternate-leaved Dogwood.
In woods throughout the region.
CORNUS OBLIQUA Raf. Kinnikinnik.
Cornus Amomum of Auth. in part not Mill.
Along banks and rivers.
CORNUS RACEMOSA Lam. Panicled Dogwood.
Cornus paniculata L'Her.
Cornus candidissima Marsh.
Cornus femina of auth., not Mill.
A common shrub in upland oak and hickory woods, sometimes on the prairie.
CORNUS RUGOSA Lam. Broad-leaved Dogwood.
Cornus circinata L'Her.
Collected in upland open woods at McGregor and Bluffton.

CORNUS STOLONIFERA Michx. Red-osier Dogwood.
In Upper Iowa River Valley on limestone rocks on shady hillside at Pulpit Rock Park and three miles west of Decorah.

ERICACEAE (Heath Family)

MONOTROPA UNIFLORA L. Indian's Pipe.
Rare in rich humus soil in linden-maple woods in Hanover Township in Allamakee County.

PYROLA ELLIPTICA Nutt. Shin-leaf.
Collected in linden-maple woods in Hanover Township and in upland oak woods at Conover Station.

PYROLA SECUNDA L. One-sided Wintergreen.
I. S. C. Herbarium 71,965. Collected by Holway near Decorah, July 2, 1893.

VACCINIUM CANADENSE Kalm. Canadian Blueberry.
Collected on Pike's Peak near McGregor in open woods of paper birch on sandy soil from St. Peter sandstone.

PRIMULACEAE (Primrose Family)

ANDROSACE OCCIDENTALIS Pursh. Androsace.
In limestone rocky soil on wind-swept hilltops at Decorah.

DODECATHEON AMETHYSTINUM (Fassett) Fassett. Cliff Shooting Star.
See Rhodora 31: 52. 1929, and Rhodora 33: 255. 1931.
Dodecatheon Meadia L. var. *amethystinum* Fassett.
In linden-maple woods on sandstone and limestone cliff on top of west hillside in Mississippi River Valley four miles north of Lansing.

LYSIMACHIA CILIATA L. Fringed Loosestrife.
See Rhodora 39. 1937.
Steironema ciliatum (L.) Raf.
In weed patches in swamps, meadows and along rivers throughout the region.

LYSIMACHIA HYBRIDA Michx. Lance-leaved Loosestrife.
See Rhodora 39. 1937.
Steironema lanceolatum (Walt.) Gray in part.
Along banks of the Mississippi River at Marquette.

LYSIMACHIA NUMMULARIA L. Moneywort.
On open hillside at Lower Dam and in soft maple woods in Mississippi River Valley at McGregor. European.

LYSIMACHIA QUADRIFLORA Walt. Linear-leaved Loosetrife.
See Rhodora 39: 1. 1937.
Steironema quadriflorum (Walt.) Gray.
Common in upland prairie meadows and swamps near Ridgeway and Calmar.

LYSIMACHIA TERRESTRIS (L.) BSP. Bulb-bearing Loosestrife.
In Mississippi Valley swamp north of Lansing.

LYSIMACHIA THYRSIFLORA L. Tufted Loosestrife.
Rare in dried up Mississippi Valley slough one mile south of Lansing.

OLEACEAE (Olive Family)

FRAXINUS CAMPESTRIS Britton. Western Green Ash.
Collected in Upper Iowa River Valley at Decorah.

FRAXINUS LANCEOLATA Borkh. Eastern Green Ash.
Fraxinus pennsylvanica lanceolata (Borkh.) Sarg.
Common in river valleys throughout the region; forms an important part of the valley woods.

FRAXINUS NIGRA Marsh. Black Ash.
Occasional tree along rivers and creeks.

FRAXINUS PENNSYLVANICA Marsh. Red Ash.
Collected on sunny hillside near Freeport.

GENTIANACEAE (Gentian Family)

GENTIANA ANDREWSII Griseb. Closed Gentian.
On swampy prairie land near Ridgeway and on edge of swamp four miles west of New Albin.

GENTIANA CRINITA Froel. Fringed Gentian.
Collected on open, south hillside on disturbed soil along new road near New Albin, and on prairie lowland near Ridgeway.

GENTIANA FLAVIDA Gray. Yellow Gentian.
Occasional on prairie along Milwaukee Railroad between Calmar and Ridgeway.

GENTIANA SAPONARIA L. Soapwort Gentian.
Rare on prairie along Milwaukee Railroad near Ridgeway.

GENTIANA PUBERULA Michx. Prairie Gentian.
Common on well drained prairie land at Calmar and Ridgeway.

GENTIANA QUINQUEFOLIA L. Stiff Gentiana.
On shady hillsides near Decorah, under *Juniper virginiana* trees in Hanover Township, and on prairie near Ridgeway.

APOCYNACEAE (Dogbane Family)

APOCYNUM ANDROSAEMIFOLIUM L. Spreading Dogbane.
On prairies and open woods.

APOCYNUM CANNABINUM L. Indian Hemp.
A weedy plant in fields and waste places.

ASCLEPIADACEAE (Milkweed Family)

ACERATES FLORIDANA (Lam.) Hitchc.
On sandy hillside in Hanover Township.

ASCLEPIAS AMPLEXICAULIS Sm. Blunt-leaved Milkweed.
On sandy soils on prairie two miles south of Decorah.

ASCLEPIAS INCARNATA L. Swamp Milkweed.
Common in swamps at Freeport, prairie swamps at Ridgeway, and in Mississippi Valley.

ASCLEPIAS OVALIFOLIA Dec. Oval-leaved Milkweed.
Rare on prairie two miles east of Calmar.

ASCLEPIAS SYRIACA L. Common Milkweed.
Common weed throughout the region.

ASCLEPIAS TUBEROSA L. Butterfly-weed.
Frequent in open woods and on prairies.

ASCLEPIAS VERTICILLATA L. Whorled Milkweed.
In sandy soil at Decorah and Hanover Township.

ASCLEPIAS EXALTATA (L.) Muhl. Poke Milkweed.
Asclepias phytolaccoides Pursh.
Collected in open woods at Lower Dam and at McGregor.

CONVOLVULACEAE (Morning-Glory Family)

CONVOLVULUS ARVENSIS L. Creeping Jenny.
In railroad yards at Decorah in well drained gravel and cinders.

CONVOLVULUS SEPIUM L. Hedge Bindweed.
Common weed in fields, prairies, and waste places.

CUSCUTA GRONOVII Willd. Love-vine.
On Golden-rod in woods at Upper Dam.

CUSCUTA GLOMERATA Chois. Glomerate Dodder.
Cuscuta paradoxa Raf.
Parasitic on golden rods on prairie at Conover.

POLEMONIACEAE (Polemonium Family)

PHLOX DIVARICATA L. Woodland Phlox.
Common in valley woods on alluvial soils and on the hillsides in linden-maple woods.

PHLOX MACULATA L. Spotted-leaf Phlox.
On swampy prairie land near Ridgeway.

PHLOX PILOSA L. Prairie Phlox.
Common plant on prairies and open woods on well drained soils.

POLEMONIUM REPTANS L. Creeping False Blue-bell.
In open bur oak woods near Decorah.

HYDROPHYLLACEAE (Waterleaf Family)

ELLISIA NYCTELEA L. Nyctelea.
In moist valley woods on alluvial soils. An annual plant making quick early growth and maturing early.

HYDROPHYLLUM APPENDICULATUM Michx. Appendaged Water-leaf.
In linden-maple woods at mouth of Canoe Creek.

HYDROPHYLLUM VIRGINIANUM L. Virginia Water-leaf.
Common spring flower on the alluvial plains and in the linden-maple woods throughout the region.

BORAGINACEAE (Borage Family)

CYNOGLOSSUM OFFICINALE L. Gipsy Flower.
In woods around farm yards in vicinity of Decorah.

LAPPULA ECHINATA Gilbert. Stickseed.
Growing in pasture on sunny hillside at Decorah.

LAPPULA REDOWSKII (Hornem.) Green var. OCCIDENTALIS (Wats.) Rydb.
I. S. C. Herbarium 92,186; collected by R. I. Cratty at Decorah, July, 1882.

LAPPULA VIRGINIANA (L.) Greene. Virginia Stickseed.
Common in open woods or in cut-over wood areas.

LITHOSPERMUM ANGUSTIFOLIUM Michx. Narrow-leaved Puccoon.
Collected in Hanover Township in Allamakee County on sandy river terrace.
LITHOSPERMUM CANESCENS (Michx.) Hitchc. Hoary Puccoon.
Common on well drained prairie lands and sunny hillsides.
LITHOSPERMUM GMELINI (Michx.) Hitchc. Hairy Puccoon.
On sandy river terrace at Freeport and four miles west of New Albin in Upper Iowa River Valley.
LITHOSPERMUM LATIFOLIUM Michx. Broad-leaved Puccoon.
In oak-hickory woods on north hillside in Pallisade Park at Decorah.
MERTENSIA PANICULATA (Ait.) G. Don. Tall Lungwort.
I. S. C. Herbarium 92,092; collected by R. I. Cratty, May 29, 1881, at Decorah.
MERTENSIA VIRGINICA (L.) Link. False Blue Bell. Lungwort.
Common in vicinity of Decorah on flood plains.
ONOSMODIUM OCCIDENTALE Mackenzie. Western Gromwell.
On sunny hillside on river terrace in Hanover Township.

VERBENACEAE (Vervain Family)

LIPPIA LANCEOLATA Michx. Frog Fruit.
On banks of sloughs in the Mississippi River Valley.
VERBENA BRACTEATA Lag. & Rodr. Blue Vervain.
See Ann. Mo. Bot. Gard. 20: 304. 1933.
Verbena bracteosa Michx.
Common weed in alluvial pastures.
VERBENA HASTATA L. Blue Vervain.
In moist location in alluvial pastures and swamps.
VERBENA SIMPLEX Lehm. Narrow-leaved Vervain.
See Ann. Mo. Bot. Gard. 20: 282.
Verbena angustifolia Michx.
Common weed in alluvial pastures.
VERBENA STRICTA Vent.
A weedy plant in pastures, roadsides, and waste places.
VERBENA URTICAEFOLIA L. White Vervain.
On edge of swamp near Freeport and in alluvial pastures.

LABIATAE (Mint Family)

AGASTACHE NEPETOIDES (L.) Ktze. Giant Hyssop.
In weed patch on edge of field in Upper Iowa River Valley four miles west of New Albin.
AGASTACHE SCROPHULARIAEFOLIA (Willd.) Ktze. Figwort.
Common in habitats similar to the above species.
BLEPHILIA HIRSUTA (Pursh.) Benth. Hairy Blephilia.
In linden-maple woods in Upper Iowa River Valley in Allamakee County and in Mississippi Xalley near McGregor.
HEDEOMA HISPIDA Pursh.
Frequent on sunny, rocky hillsides.
ISANTHUS BRACHIATUS (L.) BSP. False Pennyroyal.
On limestone rocks on sunny hillsides at Decorah.

GALEOPSIS TETRAHIT L. Hemp-nettle.
Collected at Fort Atkinson in dried up pond. European.

LEONURUS CARDIACA L. Motherwort.
Common weed around farm yards and dwellings. European.

LYCOPUS AMERICANUS Muhl. Cut-leaved Water Horehound.
Collected along stream at Fort Atkinson.

LYCOPUS VIRGINICUS L. Bugle Weed.
Collected in swamps near Ridgeway and Calmar.

MENTHA CANADENSIS L. Canadian Mint.
Common in swampy areas throughout the region.

MENTHA GENTILIS L. Creeping or Downy Whorled Mint.
In swampy land one mile north of Freeport.

MONARDA MOLLIS L. Blue Horse Mint.
Common weedy plant in open woods, pastures, prairies and along edges of fields.

MONARDA PUNCTATA L. Spotted Horse Mint.
On sandy soil of river terraces in Upper Iowa River Valley in Allamakee County.

NEPETA CATARIA L. Catnip.
Common around dwellings and barns. European.

NEPETA HEDERACEA (L.) Trevisan. Ground Ivy.
Frequent in moist valley woods.

PHYSOSTEGIA PARVIFLORA Nutt.
Common in moist, alluvial soil along ponds, sloughs, and swamps.

PRUNELLA VULGARIS L. Self-heal. Heal-all.
Common in open woods, pastures, and meadows.

PYCNANTHEMUM VIRGINIANUM (L.) Durand and Jackson. Mountain Mint.
Frequent on prairies and borders of fields.

SALVIA LANCEAEFOLIA Poir.
A weedy plant in sandy soil in pastures near Freeport.

SCUTELLARIA OVATA Hill. Heart-leaved Skullcap.
See Contr. U. S. Nat. Herb. 22: 734. 1927.
Scutellaria cordifolia Muhl.
Scutellaria versicolor Nutt.
Rare in oak-hickory woods at Lower Dam and across river from McGregor.

SCUTELLARIA LATERIFLORA L. Blue Skullcap.
Common along river banks and sloughs.

SCUTELLARIA PARVULA Michx. Small Skullcap.
Frequent on sunny, rocky hillsides.

STACHYS ASPERA Michx. Rough Hedge Nettle.
Common in weed patches in moist open woods, along edges of valley fields and roadsides.

STACHYS PALUSTRIS L. Marsh Hedge Nettle.
In swamp prairie lands near Calmar.

TEUCRIUM CANADENSE L.
Common weedy plant in barn yards, roadsides, railroad tracks.

SOLANACEAE (Nightshade Family)

DATURA TATULA L. Jimson-weed.
Growing in corn field near Union City.

DATURA STRAMONIUM L. Jimson-weed.
In barn yard near Ridgeway.
PHYSALIS HETEROPHYLLA Nees. Ground Cherry.
On sandy river terrace near Freeport.
PHYSALIS SUBGLABRATA Mackenzie and Bush.
Growing in field in Hanover Township in Upper Iowa Valley.
PHYSALIS VIRGINIANA Mill. Virginia Ground Cherry.
Common on prairies in well drained areas.
SOLANUM CAROLINENSE L. Horse Nettle.
In corn field in Upper Iowa River Valley.
SOLANUM DULCAMARA L. Climbing Nightshade.
Common vine in yards and gardens. European.
SOLANUM NIGRUM L. Black Nightshade.
Common weed in waste places, along roads and in gardens.
SOLANUM ROSTRATUM Dunal. Buffalo-bur. Prickly Nightshade.
On sandy river terrace in Hanover Township near farm yard.

SCROPHULARIACEAE (Figwort Family)

GERARDIA ASPERA Dougl. Rough Purple Gerardia.
See Pennell Scroph. of E. Tem. N. A., p. 428. 1935.
Agalinis asper (Dougl.) Britton.
On sunny, north hillsides in Mississippi River Valley north of Lansing.
GERARDIA TENUIFOLIA var. MACROPHYLLA (Benth.) Blake.
See Pennell Scroph. of E. Tem. N. A., p. 462. 1935.
GERARDIA TENUIFOLIA var. MACROPHYLLA (Benth.) Blake.
Common in swampy areas on prairie near Ridgeway and in Mississippi and Upper Iowa River Valleys.
CASTILLEJA COCCINEA (L.) Spreng. Indian Paint Brush.
I. S. C. Herbarium 38,553; collected by Holway, May 21, 1881, near Decorah. I. S. C. Herbarium 72,261; collected by Goddard, May 9, 1895, near Decorah.
CASTILLEJA SESSILIFLORA Pursh. Prairie or Downy Painted-cup.
On sunny rocky hillside at Decorah.
CHELONE GLABRA L. Snake-head. Turtle-head.
In swamp near Freeport and along shores of rivers.
ILYSANTHES DUBIA (L.) Barnhart. Long-stalked False Pimpernel.
Common on muddy banks of Upper Iowa River and Mississippi sloughs.
GRATIOLA NEGLECTA Torrey. Hedge Hyssop.
See Pennell Scroph. of E. Tem. N. A., p. 428. 1935.
Rhodora 34: 147. 1932.
Gratiola lutea glaberrima Fernald.
Along banks of a slough in Mississippi River Valley near New Albin.
LINARIA MINOR Desf. Small Snap Dragon.
Chaenorrhinum minus (L.) Lange.
Along railroad track near McGregor in gravelly and ashy soil.
LINARIA VULGARIS Hill. Butter and Eggs.
In waste places throughout the region.

MIMULUS RINGENS L.
In Mississippi River Valley swamps and along rivers throughout the region.
PEDICULARIS CANADENSIS L. Wood Betony.
In woods on south hillside near Decorah and on prairie lowland three miles north of Oelwein.
PEDICULARIS LANCEOLATA Michx. Swamp Lousewort.
In swamp near Freeport and four miles west of New Albin.
SCROPHULARIA LANCEOLATA Pursh. Bare Figwort or Leopard Figwort.
See Torreya 22: 81-84. 1922.
Scrophularia leporella Bicknell.
In weed patches in Upper Iowa River Valley and on prairies.
SCROPHULARIA MARILANDICA L. Figwort.
In weed patches in Upper Iowa River Valley.
VERBASCUM THAPSUS L. Mullein.
Common weed in pastures.
VERONICA ANAGALLIS-AQUATICA L. Water Speedwell.
In water of outlet at springs around Decorah.
VERONICA PEREGRINA L. Purslane Speedwell.
On edges of river and sloughs in moist alluvial soil.
VERONICASTRUM VIRGINICUM (L.) Farwell. Culver's-root.
See Drug Circ. 61: 231. 1917.
Veronica virginica L.
Frequent on prairies.

BIGNONIACEAE (Bignonia Family)

CATALPA SPECIOSA Warder. Large Catalpa.
Planted in lawns at Decorah.
CATALPA BIGNONIOIDES Walt. Small Catalpa. Indian Smoking Bean.
Planted in lawns at Decorah.

PHRYMACEAE (Lopseed Family)

PHRYMA LEPTOSTACHYA L. Lopseed.
Frequent in oak woods throughout the region.

PLANTAGINACEAE (Plantain Family)

PLANTAGO MAJOR L. Large Plantain.
A common weed in lawns and similar locations.
PLANTAGO RUGELII Dcne.
In habitats similar to *P. major*.

RUBIACEAE (Madder Family)

CEPHALANTHUS OCCIDENTALIS L. Button-bush. Globe-flower.
Along banks of Mississippi River at Marquette and McGregor.
GALIUM APARINE L. Cleavers.
Common vernal species in valley woods.
GALIUM ASPRELLUM Michx. Rough Bedstraw.
In swamp near Freeport and four miles west of New Albin.
GALIUM BOREALE L. Northern Bedstraw.
Common on prairies and open woods.

GALIUM CIRCAEZANS Michx. Cross-cleavers.
In linden-maple woods in vicinity of McGregor.
GALIUM CONCINNUM T. & G. Shining Bedstraw.
Frequent in open woods.
GALIUM TINCTORIUM L. Stiff Marsh Bedstraw.
In swamp two miles south of Lansing and in soft maple woods along Mississippi Valley slough in vicinity of McGregor.
GALIUM TRIFLORUM L. Fragrant Bedstraw.
Common in linden-maple woods of the area.

CAPRIFOLIACEAE (Honeysuckle Family)

ADOXA MOSCHATELLINA L. Musk-root.
In linden-maple woods, usually at bottom of tree trunks where dead leaves do not lodge.
DIERVILLA LONICERA Mill. Bush Honeysuckle.
Common on shady hillsides usually in open woods.
LINNAEA BOREALIS L. var. AMERICANA (Forbes) Rehder. Twin Flower.
I. S. C. Herbarium 36,061; collected by E. H. Case at Decorah, June 19, 1893.
LONICERA DIOICA var. GLAUCESCENS (Rydb.) Butters. Bush Honeysuckle.
See Rosendahl and Butters, Minnesota Trees and Shrubs.
L. glaucescens Rydb.
In open woods on south hillside at Decorah.
LONICERA PROLIFERA (Kirchner) Rehder. Bush Honeysuckle.
Common on calcareous cliffs and rocks and open woods.
LONICERA SEMPERVIRENS L. Ever-living Honeysuckle.
Occasionally planted on lawns.
LONICERA TATARICA L. Siberian Honeysuckle.
A common escape from cultivation in open woods and pastures in the vicinity of Decorah.
SYMPHORICARPOS ORBICULATUS Moench. Red Wolf-berry.
Planted in lawns at Decorah.
SYMPHORICARPOS OCCIDENTALIS Hook. Western Wolf-berry.
Collected on south, sunny hillside at Bluffton and on railroad bank near Ossian.
TRIOSTEUM AURANTIACUM Bicknell. Horse Gentian.
On north hillsides in open woods of burr oak and hickory.
TRIOSTEUM PERFOLIATUM L.
In habitats similar to *T. aurantiacum.*
SAMBUCUS CANADENSIS L. Large or Canadian Elder-berry.
Common in thickets and open woods in river bottoms.
SAMBUCUS PUBENS Michx. Red Elder Berry.
See Fl. Bor. Am. 1: 181. 1803.
Sambucus racemosa of auth., not Pursh.
Common in linden-maple and elm-ash woods of flood plains.
VIBURNUM AFFINE Bush. Smooth-leaved Viburnum.
See Rhodora 20: 14. 1918.
V. pubescens of auth., not Pursh.
Frequent in upland oak woods.
VIBURNUM AFFINE Bush. var. HYPOMALACUM Blake. Pubescent-leaved Viburnum.

See Rhodora 20:14. 1918.
On stage road near Waukon, Allamakee County.

Viburnum Lentago L. Nanny-berry.
Frequent in open valley woods.

Viburnum trilobum Marsh. Cranberry-bush.
V. Opulus var. *americanum* (Mill.) Ait.
Rare in linden-maple woods near Decorah and in Canoe Creek Valley six miles north of Decorah.

VALERIANACEAE (Valerian Family)

Valeriana edulis Nutt. Valerian. Tobacco-root.
On east, sunny hillside two miles southeast of Decorah.

CUCURBITACEAE (Gourd Family)

Echinocystis lobata (Michx.) T. & G. Balsam Apple. Mock Apple.
In thickets in moist places and alluvial soil along rivers.

CAMPANULACEAE (Bluebell Family)

Campanula americana L. American Bluebell.
Common in weed patches in valley woods along rivers.

Campanula aparinoides Pursh. Swamp Bluebell.
In swampy land at small spring in Hanover Township.

Campanula rapunculoides L. European Creeping Bluebell.
An escape from cultivation in disturbed areas along road near Burr Oak.

Campanula rotundifolia L. Harebell. Round-leaved Bluebell.
Common on limestone rocks and cliffs and well drained sunny hillsides.

Campanula uliginosa Rydb.
Growing on moist hillside under red oak trees in Upper Iowa Valley in Allamakee County.

Specularia perfoliata (L.) A. DC. Venus' Looking-glass.
In open pasture near Lansing and at Upper Dam in sandy soils.

LOBELIACEAE (Lobelia Family)

Lobelia cardinalis L. Cardinal Flower.
In open woods on heavy, muddy soils in Mississippi Valley near Marquette.

Lobelia inflata L. Indian Tobacco.
In open woods at small spring in Hanover Township.

Lobelia siphilitica L. Blue Lobelia.
Common in swamps, ditches, and along banks of rivers.

Lobelia spicata Lam. Small Flowered Prairie Lobelia.
In lowland meadows on prairie near Calmar and at Lower Dam.

COMPOSITAE (Sunflower Family)

Achillea lanulosa Nutt. Woolly Yarrow.
In sandy soil at New Albin along Milwaukee Railroad.

ACHILLEA MILLEFOLIUM L.
Common on prairies, pastures, and lawns.
ANTENNARIA NEGLECTA Greene. Field Cat's Foot.
Common in open woods and on prairies.
ANTENNARIA PLATAGINIFOLIA (L.) Richards. Wide-leaved Cat's Foot.
Frequent in open woods.
AMBROSIA ARTEMISIAEFOLIA var. ELATIOR (L.) Desc. Small Ragweed.
A. artemisiaefolia of Auth., not L.
Common weed throughout the region.
AMBROSIA PSILOSTACHYA DC. Western Ragweed.
At New Albin on sandy soil along Milwaukee Railroad.
AMBROSIA TRIFIDA L. Great Ragweed.
Common weedy plant in moist locations, especially low areas recently covered with mud by floods.
ANTHEMIS COTULA L. Barn-yard Aster.
A weedy European plant common around barn yards.
ARCTIUM MINUS Bernh. Common Burdock.
In open woods near college at Decorah.
ARTEMISIA ABSINTHIUM L. Absinth.
An European plant found in open, rocky pasture in valley near Decorah.
ARTEMISIA BIENNIS Willd. Biennial Wormwood.
In disturbed soil along roads.
ARTEMISIA CAUDATA Nutt. Tall Wormwood.
In sandy soils on river terraces near Freeport.
ARTEMISIA DRACUNCULOIDES Pursh. Linear-leaved Wormwood.
I. S. C. Herbarium specimen. Collected by Herbert Goddard August 14, 1899, in Winneshiek County.
ARTEMISIA VULGARIS var. GNAPHALODES (Nutt.) Ktze. Prairie Mugwort.
Common on prairies and open, sunny woods. Increasing by colonies and indicator of over grazing in pastures.
ARTEMISIA SERRATA Nutt. Saw-leaved Mugwort.
In weedy thickets along fields and roads in Upper Iowa River Valley. Collected near Decorah and Upper Dam.
ASTER AZUREUS Lindl. Sky-blue Aster.
Common on prairies.
ASTER DRUMMONDII Lindl. Drummond's Aster.
Frequent in open woods throughout area.
ASTER CORDIFOLIUS L. Common Blue Wood Aster. Heart-leaved Aster.
In linden-maple woods at Canoe Creek.
ASTER ERICOIDES L. Many-flowered Aster.
See Rhodora 32: 138. 1930; Rhodora 30: 227. 1928.
Aster multiflorus Ait.
Common on prairies and open, sunny hillsides.
ASTER INTERIOR Wiegand.
See Rhodora 35: 35, 312. 1933.
Aster Tradescanti of auth., not L.
Common on prairie lowlands and along creeks.
ASTER LAEVIS L. Smooth Aster.
Common on prairies throughout region.

ASTER NOVAE-ANGLIAE L. New England Aster.
Common on prairie lowlands and along roads in ditches.

ASTER PRAEALTUS Poir. Willow-leaved Aster.
See Rhodora 35: 21. 1933.
Aster salicifolius Ait.
Common on prairie lowlands and along creeks.

ASTER PRENANTHOIDES Muhl. Crooked-stem Aster.
Collected in swampy area in Canoe Creek Valley.

ASTER PTARMICOIDES (Nees.) T. & G. Prairie White Aster.
On sunny hillside three miles north of New Albin.

ASTER PUNICEUS L. Purple-stem Aster.
In ditch along roadside near Decorah.

ASTER SAGITTIFOLIUS Willd. Arrow-leaved Aster.
In open woods near Decorah.

ASTER SERICEUS Vent. Western Silky Aster.
On glacial till in railroad cut near Calmar and on calcareous rocky soil on north, sunny hillside at Union City.

ASTER SHORTII Lindl. Short's Aster.
In linden-maple woods at Bluffton and Decorah.

ASTER UMBELLATUS Mill. Flat-top Aster.
On lowland prairie at Conover and in swamp near New Albin.

BIDENS CERNUA L. Nodding Bur-marigold.
Common along streams and in swamps throughout the area.

BIDENS CONNATA Muhl. Purple-stemmed Swamp Beggar-ticks.
Collected along Canoe Creek.

BIDENS COMOSA (A. Gray) Wiegand. Leafy-bracted Tickseed.
Collected along Canoe Creek.

BIDENS FRONDOSA L. Beggar Ticks.
Collected in swampy land along Canoe Creek. Often growing as a weed in valley fields.

BIDENS VULGATA Greene. Tall Beggar-ticks.
In swampy meadow two miles north of Lansing.

BOLTONIA ASTEROIDES (L.) L'Her. False Starwort.
Common in swampy land in Mississippi Valley.

CACALIA RENIFORMIS Muhl. Great Indian Plantain.
Collected in linden-maple woods at Bluffton and in open ash-elm woods near Decorah on alluvial flood plain.

CACALIA TUBEROSA Nutt. Tuberous Indian Plantain.
On prairie hillside in Pulpit Rock Park at Decorah.

CHRYSANTHEMUM LEUCANTHEMUM L. White-eyed Daisy.
A weedy plant in pastures and hay fields.

CICHORIUM INTYBUS L. Wild Succory.
Weedy plant along roadsides and waste places.

CIRSIUM ALTISSIMUM (L.) Spreng. Tall Thistle.
See Beih. z. Bot. Centralbl. 35: 396. 1917.
Including *Cirsium iowense* (Pammel) Fernald.
In swampy land at spring one mile north of Freeport.

CIRSIUM ARVENSE (L.) Scop. Canadian Thistle.
Common weed in fields causing considerable trouble to farmers.

CIRSIUM DISCOLOR (Muhl.) Spreng. Field Thistle.
In pastures and waste places.

CIRSIUM LANCEOLATUM (L.) Hill. Common Bur Thistle.
In pastures and waste places.
CIRSIUM ODORATUM (Muhl.) Petrak. Hill's Thistle.
See Beih. z. Bot. Centralbl. 35: 381. 1917.
Cirsium Hillii (Canby) Fernald.
On prairie near Conover along Milwaukee Railroad.
COREOPSIS PALMATA Nutt. Palmate-leaved Coreopsis.
Common on prairies and open, sunny hillsides throughout the region.
ERECHTITES HIERACIFOLIA (L.) Raf. Fire-weed.
In swamps four miles west of New Albin.
ERIGERON ANNUUS (L.) Pers. Annual Fleabane. White Top.
Common in fields.
ERIGERON CANADENSIS L. Canadian Erigeron or Fleabane.
Weedy plant in fields and gardens.
ERIGERON PHILADELPHICUS L. Woodland Fleabane.
Common in valleys on alluvial soil.
ERIGERON PULCHELLUS Michx. Robin's Plantain.
Frequent in open woods throughout the region.
ERIGERON RAMOSUS (Walt.) BSP. Daisy Fleabane.
In fields and open waste places.
EUPATORIUM ALTISSIMUM L. Thoroughwort.
On sandy river terrace at Upper Dam.
EUPATORIUM MACULATUM L. Spotted-leaved Joe-Pye Weed.
Found occasionally in valley woods.
EUPATORIUM PERFOLIATUM L. Boneset.
In swamps near Freeport and New Albin.
EUPATORIUM PURPUREUM L. Joe-Pye Weed.
In swamp near Freeport and Marquette.
EUPATORIUM URTICAEFOLIUM Reichard. White Snakeroot.
Common in linden-maple woods.
GALINSOGA PARVIFLORA Cav. Galinsoga.
In shady, moist locations at Lansing.
GNAPHALIUM POLYCEPHALUM Michx.
Four miles west of New Albin in Allamakee County.
GRINDELIA SQUARROSA Dunal. Gum Plant.
In gravelly, well drained pasture near Decorah and Lansing.
HELENIUM AUTUMNALE L. Swamp Sunflower.
In alluvial pastures and swamps.
HELIANTHUS ANNUUS L. Annual Sunflower.
At City Dump of New Albin.
HELIANTHUS OCCIDENTALIS Riddell. Western Sunflower.
On prairie hillsides on well drained, calcareous rocky soils in Hanover Township.
HELIANTHUS PETIOLARIS Nutt.
In gravel pit near New Albin.
HELIANTHUS SCABERRIMUS Ell. Stiff Sunflower.
Common on dry prairies.
HELIANTHUS TUBEROSUS L. Jerusalem Artichoke.
Common in thickets and edges of woods.

Heliopsis scabra Dunal. Rough Ox-eye.
Common on prairies.

Hieracium canadense Michx. Canadian Hawkweed.
In low prairie land near Ridgeway.

Hieracium longipilum Torr.
On sandy river terrace four miles west of New Albin in Upper Iowa River Valley and in open hay land bordered by woods near McGregor.

Hieracium scabrum Michx. Rough Hawkweed.
In oak woods on south hillside, Hanover Township.

Inula Helenium L. Horseheel.
Collected at Decorah. Rare.

Iva xanthifolia Nutt. Burweed Marsh Elder.
A tall, weedy plant in barnyards and similar places.

Krigia biflora (Walt.) Blake. Virginia Goat's Beard.
See Rhodora 17: 135. 1915.
Krigia amplexicaulis Nutt.
In linden-maple woods at McGregor and in low, swampy meadow at Upper Dam.

Kuhnia eupatorioides L. False Boneset.
On sandy soils of river terrace and sunny prairie hillsides.

Lactuca canadensis L. Canadian Wild Lettuce.
Four miles west of New Albin in Upper Iowa River Valley.

Lactuca scariola L. Prickly Lettuce.
Common weed in disturbed land throughout the area.

Lactuca spicata (Lam.) Hitchc. Tall Blue Lettuce.
Along roadside in open woods, Upper Iowa River Valley, Winneshiek County.

Lactuca villosa Jacq. Hairy Veined Blue Lettuce.
In linden-maple woods in Hanover Township in Allamakee County.

Lepachys pinnata T. & G. Yellow Coneflower.
Common on tall grass prairies near Calmar.

Liatris cylindracea Michx. Cylindric Blazing Star.
On sunny hillsides in Hanover Township.

Liatris pycnostachya Michx. Hairy Blazing Star.
On moist prairies between Calmar and Ridgeway.

Liatris scariosa Willd. Scarious-bracted Blazing Star.
In habitats similar to the above species.

Parthenium integrifolium L. Prairie Dock.
On prairies between Calmar and Ridgeway.

Polymnia canadensis L. Small-flowered Leaf Cup.
Common in linden-maple woods throughout area.

Prenanthes alba L. Rattlesnake Root.
In linden-maple woods throughout area.

Prenanthes racemosa Michx. Glaucous White-lettuce.
Common on prairies between Ridgeway and Calmar.

Rudbeckia hirta L. Black-eyed Susan.
Common on prairies, hayfield, and open woods.

Rudbeckia laciniata L. Green-headed Cone-flower.
Common in alluvial soil in valley woods.

RUDBECKIA TRILOBA L. Thin-leaved Cone-flower.
Common in woods along rivers and creeks.

SENECIO PLATTENSIS Nutt. Prairie Ragwort.
On sandy soil of river terraces south of Freeport.

SENECIO AUREUS L. Golden Ragwort, Swamp Squaw-weed.
On shores of Canoe Creek in moist, alluvial soil, and at spring near Upper Dam.

SENECIO BALSAMITAE Muhl. Balsam Groundsel.
On sandy river terrace. Winneshiek County.

SILPHIUM LACINIATUM L. Compass-plant.
On upland prairies.

SILPHIUM PERFOLIATUM L. Indian Cup.
In low areas along roads and along rivers.

SONCHUS ARVENSIS L. var. ULIGINOSUS Beib.
Growing in city dump at Decorah.

SONCHUS OLERACEUS L. Annual Sow Thistle.
Growing along railroad at McGregor.

SOLIDAGO CAESIA L. Blue Stemmed Goldenrod.
In Upper Iowa River Valley on limestone cliffs and outcrops near Decorah and Union City.

SOLIDAGO CANADENSIS L. Canadian Goldenrod.
Common on prairies, open valleys and meadows.

SOLIDAGO GRAMINIFOLIA (L.) Salisb.
Euthamia graminifolia (L.) Nutt.
On prairies at Calmar and Ridgeway.

SOLIDAGO LATIFOLIA L. Broad-leaved Goldenrod.
Common in linden-maple woods.

SOLIDAGO GLABERRIMA Martens. Smooth Goldenrod.
S. missouriensis A. Gray; not Nutt.
On upland prairies.

SOLIDAGO NEMORALIS Ait. Gray Goldenrod.
On upland prairies and sunny rocky hillsides.

SOLIDAGO SEROTINA Ait. Serrate-leaved Goldenrod.
Common in weedy thickets along fields and waste places.

SOLIDAGO SPECIOSA Nutt. var. ANGUSTATA T. & G.
On upland open woods and on prairies.

SOLIDAGO RIDDELLII Frank. Riddell's Swamp Goldenrod.
In low swampy land on prairie near Ridgeway.

SOLIDAGO RIGIDA L. Stiff Goldenrod.
Common on upland prairie.

SOLIDAGO ULMIFOLIA Muhl. Elm-leaved Goldenrod.
Common in woodlands throughout the area.

TANACETUM VULGARE L. Tansy.
An escape near farmhouses in Winneshiek County.

TARAXACUM OFFICINALE Weber. Dandelion.
Common weed in lawns, waste places throughout the region.

TRAGOPOGON PRATENSIS L. Goat's Beard.
On disturbed lands along roadsides.

VERONICA FASICULATA Michx. Ironweed.
Frequent on alluvial soil.

PLANT FORMATIONS

Two plant formations are common in Winneshiek and Allamakee counties. The tall grass association of the grassland formation is found on the prairie ridges and on the xeric hillsides. The deciduous forest formation is represented in the valleys and extends far up into the prairie highlands. A third formation, the Great Lakes Coniferous Forest, is represented by scattered groves of coniferous trees.

THE HYDROSERE

Winneshiek and Allamakee counties have few lakes, ponds, or swamps because of a well developed drainage system. The successive elevations of this region without base leveling have resulted in swift-flowing rivers and creeks. For this reason hydrophytic vegetation is poorly represented, except along the Mississippi River and to a lesser extent along its tributaries, and in the vicinity of an occasional spring outlet.

In the stagnant, slow-moving water of the Mississippi sloughs and ponds the chief submerged forms include those of *Ceratophyllum demersum* and species of *Myriophyllum* and *Potomogeton.* The floating plants include *Lemna* and *Wolffia* which occur with *Nymphaea odorata, Nuphar advena* and species of *Potomogeton.* Emerging from the water along the muddy shores are dense communities of *Sparganium eurycarpum, Scirpus validus, Sagittaria latifolia*, and *Nelumbo lutea.* Hydrophytic vegetation in the Upper Iowa, Yellow, and Turkey rivers is sparse because of the swift currents and frequent disturbance by heavy floods.

The cold spring waters and swamps in Canoe Creek and Upper Iowa River valleys present a distinctive flora. Submerged and floating in the cold waters are *Roripa nasturtium-aquaticum, Veronica Anagallis-aquaticum*, and *Ranunculus trichophyllus.* On the edges of these small spring streams are *Symplocarpus foetidus, Caltha palustris, Carex hystericina, Cardamine bulbosa, Acorus Calamus, Iris virginica, Impatiens pallida, Impatiens biflora, Scirpus validus*, and *Typha latifolia.* Shrubby species present are *Salix discolor, Salix cordifolia*, and *Alnus incana.*

The Carex associes is found on the poorly drained prairie meadows and borders of old lakes and ponds in the Mississippi Valley, but it is generally lacking along the rivers and small spring swamps because elevation is rapid on either side. Some prominent Carex species are *Carex hystericina, Carex lanuginosa, Carex vulpinoidea, Carex stipata*, and *Carex stricta.* Also large colonies of *Spartina pectinata* are found in low swampy meadows. On the alluvial plains the stages in primary succession beyond the Carex associes are concerned with shrubby and tree species.

Denuded areas are common along rivers where floods have eroded away river banks and formed deposits. On these denuded areas open to renewed secondary succession the first plants to enter are annual herbaceous or rapidly maturing perennial species. Among the latter are certain species of willows as *Salix interior, Salix cordifolia*, and *Populus deltoides.* Among the former are *Polygonum lapathifolium, P. pennsylvanicum, Bidens cernua, Eragrostis hypnoides, Cyperus erythrorhizos, Roripa hispida* var. *glabrata, Leersia oryzoides, Cardamine pennsylvanica*, and others. In time this annual stage may be taken over by certain perennial

woody species such as the *Salix* pioneer species or weedy species as *Boltonia asteroides, Rumex crispus, Rumex verticillata, Rumex altissimus, Physostegia parviflora, Helenium autumnale, Vernonia fasciculata* and *Lysimachia cilianensis.*

A secondary associes which follows the pioneer vegetation is made up of tall trees as *Salix amygdaloides, Salix alba, Salix nigra, Populus balsamifera,* and *Acer saccharinum.* The latter two species are longer lived than the others and frequently are found growing in the Ulmus-Fraxinus associes.

Stability is reached on the alluvial plain when the areas are dominated by an associes of *Ulmus fulva, Ulmus americana,* and *Fraxinus lanceolata.* Other trees occurring on the flood plains are *Ulmus racemosa, Celtis occidentalis, Juglans cinerea, Juglans nigra, Acer Negundo,* and *Fraxinus nigra.* Shrubs frequenting these woods are *Sambucus pubens, Sambucus canadensis, Ribes missouriensis, Evonymus atropurpurea, Rubus occidentalis,* and *Zanthoxylum americanum.* Herbaceous forms are *Erythronium albidum, Claytonia virginica, Isopyrum biternatum, Phlox divaricata, Galium Aparine, Laportea canadensis, Ellisia Nyctelea, Viola sororia, Viola eriocarpa, Rudbeckia laciniata, Rudbeckia triloba, Polygonum virginianum, Hydrophyllum virginianum, H. appendiculatum, Cacalia reniformis, Mertensia virginica, Allium tricoccum.*

THE XEROSERE

The flora of the rocks of the area consists chiefly of lichens, mosses, ferns, and a few seed plants. The rocks on the north hillsides bear a group of crustose lichens and xeric mosses. Two xeric ferns, *Pellaea glabella* and *Cheilanthes Feei,* live in crevices of sandstone and limestone cliffs. Among the higher plants on the rocks are *Sullivantia renifolia, Campanula rotundifolia, Aquilegia canadensis, Arabis lyrata,* and *Solidago caesia.* Shrubs or trees found on the limestone and sandstone cliffs include *Juniperus virginiana, Juniperus communis, Pinus Strobus, Amelanchier humilis,* and *Betula papyrifera.*

On the rocks of the south, wooded hillsides are xeric lichens and mosses, but mesic forms are more common. Great mats of foliose lichens, mosses and ferns are prominent. Among the ferns may be mentioned *Cyptogramma Stelleri, Cysptopteris bulbifera, Polypodium virginianum, Thelypteris Robertiana, Woodsia ilvensis* and *Camptosorus rhizophyllus.*

On the rocky, well-drained, north hillsides and on the well-drained prairie ridges the common, dominating grasses are *Bouteloua cultipendula,* and *Andropogon scoparius.* Other plants are *Carex pennsylvanica, Viola pedata, Anemone patens* var. *Wolfgangiana, Oxalis violacea, Lithospermum canescens, Aquilegia canadensis,* and *Ranunculus fascicularis.*

In the Upper Iowa River Valley on very sandy soil of river terraces the dominant vegetation is *Bouteloua hirsuta, Sporobolus cryptandrus,* and *Leptoloma cognatum.* In addition are a number of species rather unusual for the region. Among these are *Panicum perlongum, Froelichia floridana, Paspalum straineum, Monarda punstata, Callirhoe triangulata, Lithospermum Gmelini,* and *Hieracium longipilum.*

The tall grasses are found on the black, deep soils of the rolling prairie. Typical examples of these grasslands are found along the railroad track on the ridge between Ossian and Ridgeway. The most im-

portant species are *Andropogon furcatus, A. scoparius, Panicum virgatum, Stipa comata, Koeleria cristata,* and *Sorghastrum nutans.*

During the spring the flora of the prairie is inconspicuous. It is only toward the end of May that any conspicuous flowers bloom. The greatest floral display occurs in the fall months when the compositae are at their height.

The common herbaceous species characteristic of the prairie are listed here according to their season of blooming. Vernal species: *Anemone quinquefolia, Hypoxis hirsuta, Antennaria neglecta, Antennaria plantaginifolia, Lithospermum canescens, Viola pedata, Viola pedatifida, Oxalis violacea, Sisyrinchium campestre,* and *Pedicularis canadensis.*

Estival series: *Baptisia bracteata, Baptisia leucantha, Desmodium canadense, Achillea Millefolium, Phlox maculata, Lilium michiganense, Lilium philadelphicum,* var. *andinum, Zizia cordata, Poa pratensis, Poa compressa, Cypripedium pubescens, Koeleria cristata, Stipa spartea, Polygala Senega, Rudbeckia hirsuta, Comandra umbellata, Oxybaphus nyctagineus, Phlox pilosa, Silene stellata, Anemone canadensis, Thalictrum dasycarpum, Potentilla arguta, Potentilla canadensis, Lathyrus venosus, Petalostemum purpureum, Coreopsis palmata.*

Autumnal species: *Solidago canadensis, Solidago graminifolia, Solidago nemoralis, Solidago speciosa, Solidago rigida, Helianthus occidentalis, Helianthus scaberrimus, Helianthus grosseserratus, Gentiana flavida, Gentiana puberula, Gentiana Andrewsii, Ascepias verticillata, Prenanthes racemosus, Aster laevis, Aster azureus, Aster multiflorus, Aster novae-angliae, Aster praealtus,* and *Andropogon furcatus.*

Shrubs are common throughout the prairie. In the lower lands are several species of willow, of which *Salix discolor* is most common. Larger willow species are *Salix amygdaloides, Salix interior,* and *Salix cordifolia. Salix lucidus, Salix tristis,* and *Salix petiolaris* are found only in a few locations. Only one shrubby species of *Leguminosae, Amorpha fruticosa,* frequents the lowlands. On the higher prairie lands are found several species of shrubby plants, including *Salix humilis, Amorpha canescens, Rhus Toxicodendron, Rhus glabra, Ceanothus americanus, Rosa arkansana, Corylus americana, Cornus paniculata,* and *Spiraea alba.* Solitary trees and thickets occur frequently on the prairie. The trees in these communities are often short lived and mature rapidly. Among them are *Acer Negundo, Prunus pennsylvanica, Prunus americana,* and *Prunus virginiana.* These species are the pioneers of the advancing deciduous forests. They increase in frequency and density from the representative prairie areas to the wooded areas. When the deciduous forest is cut these species again spring up in the course of secondary succession.

The shrubby and pioneer trees grade into woods dominated by various oak species, with *Quercus macrocarpa* on the outer border. In the older woods and those of the north valley hillsides *Carya ovata* is frequently associated with *Quercus macrocarpa.* On protected uplands the *Quercus macrocarpa* and *Carya ovata* association grades into woods dominated by *Quercus alba, Quercus velutina,* and *Quercus borealis maxima.*

The herbage of the oak and hickory woods, especially along the outer border, includes species common to the prairies. The following species of shrubby plants usually are found in the oak-hickory woods: *Amelanchier canadensis, Malus ioensis, Lonicera prolifera, Prunus nigra, Prunus*

americana, Zanthoxylum americanum, Ribes missourensis, and *Celastrus scandens.*

Herbaceous species frequenting the oak-hickory woods include: *Carex pennsylvanica, Poa pratensis, Comandra umbellata, Zizia aurea, Phlox pilosa, Lithospermum canescens, Lithospermum latifolium, Triosteum perfoliatum, Polemonium reptans, Hedeoma hispida, Viola pedatifida, Apocynum androsaemifolium, Rudbeckia hirta, Anemone canadense, Desmodium canadensis, Anemonella thalictroides, Hypoxis hirsuta, Solidago ulmifolia, Potentilla canadensis. Agrimonia gryposepala, Podophyllum peltatum, Ranunculus fascicularis, Aquilegia canadensis, Draba caroliniana. Lathyrus ochroleucus, Sisyrinchium campestre, Euphorbia corollata, Hypericum punctatum, Geranium maculatum, Triosteum perfoliatum.*

The woods composed of *Quercus alba, Quercus velutina,* and *Quercus borealis maxima* form a denser shade than those where *Quercus macrocarpa* and *Carya ovata* dominate. For this reason many species of shrubs and herbs occur in the former but not in the latter woods. The shade tolerant shrubs typical of these woods are *Viburnum affine, Viburnum Lentago, Corylus americana, Rubus occidentalis, Rubus* sp., *Cornus paniculata,* and *Aralia nudicaulis.* Herbs frequenting these woods are: *Pteridium latisculum, Athyrium angustum, Podophyllum peltatum, Solidago ulmifolium, Desmodium acuminatum, Polemonium reptans, Erigeron pulchellum, Circaea latifolia, Pyrola elliptica, Oakesia sessilifolia, Smilacina racemosa, Polygonatum commutatum, Aster Drummondii, Aster Shortii, Helianthus tuberosus, Rudbeckia hirta.*

The linden and maple woods are found in the valleys and on the hillsides having deep, well developed soils. The dominant trees are *Acer nigrum, Acer saccharum,* and *Tilia glabra.* Among other trees commonly found are *Juglans cinerea, Ulmus americana, Celtis occidentalis,* and *Fraxinus lanceolata.* Under these tall trees a layer of small trees of two species, *Carpinus caroliniana* and *Ostrya virginiana*. is developed. Below these there is a layer of shrubs of which the chief species are *Staphylea trifolia, Sambucus pubens, Aralia nudicaulis, Taxus canadensis,* and rarely *Acer spicatum, Hamamelis virginiana, Dirca palustris,* and *Viburnum trilobum.*

In the herbaceous layer are found a large number of prevernal and vernal species, a few autumnal forms, and a very few festival species. The chief prevernal and vernal species are: *Actaea alba, Actaea rubra, Adoxa Moschatellina, Allium tricoccum, Anemone quinquefolia, Aralia racemosa, Asarum canadense, Arisaema triphyllum, Carex albursina, Carex grisea, Carex longirostris, Carex pedunculata, Dicentra canadensis, Dicentra cucullaria, Caulophyllum thalictroides, Claytonia virginica, Erythronium albidum, Festuca obtusa, Hepatica acutiloba, Isopyrum biternatum, Sanguinaria canadensis, Thalictrum dioicum, Trillium Gleasoni, Trillium nivale, Uvularia perfoliata, Viola eriocarpa, Viola sororia.*

Species which bloom in middle summer are: *Panax quinquefolia, Aralia racemosa,* and *Cacalia renifolia.*

The chief late summer and fall blooming plants are: *Solidago latifolium, Prenanthes alba. Eupatorium urticaefolium, Gentiana quinquefolia, Impatiens biflora, Impatiens pallida.*

SUMMARY

The total number of species collected in this survey was 846, among which were 33 species of Musci, 31 Pteridophyta, 81 Gramineae, 40 Cyperaceae, 39 Rosaceae, 37 Leguminosae, 20 Umbelliferea, 25 Labiatae, and 108 Compositae.

Two new species were recorded for the state: *Oenothera perennis* and *Woodsia scopulina*. A number of new records were made for the region.

The deciduous forest formation covers approximately three-fourths of the region and the remainder is occupied by the prairie grass formation. The forests cover all hilly regions and extend into the relatively level uplands. *Acer saccharum nigrum*, *Acer saccharum* and *Tilia glabra* are climax species; *Quercus macrocarpa* and *Carya ovata*, sub-climax species.

The prairie grasses occupy the dry hillsides and prairie ridges. In the shallow soils and wind-swept xeric hillsides and ridges the dominant grasses are *Andropogon scoparius* and *Bouteloua curtipendula*. On the prairies with deep soils and rolling topography the dominant species are *Andropogon furcatus, Sorghastrum nutans, Koeleria cristata,* and *Stipa spartea.*

The Great Lakes coniferous forest is represented by small groves of *Pinus Strobus* and *Abies balsamea.*

There are a number of species represented in the flora which are on the border of their range. These are from three geographical regions, the Great Lakes region, the western short grass prairie, and the south and southeastern prairie and forest. Three endemic species have been recorded from the region.

During recent times most of the virgin forest has been cut and both forest and prairie uplands have been plowed. Most of the land not useful for growing crops is used for pasture. Species once common are approaching extinction because of the destruction of their natural habitats. Approximately 75 species introduced from foreign lands were collected.

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EXPLANATION OF PLATES

PLATE I

No. 1. Valley hillside near Union City in Allamakee County covered with *Juniperus virginiana.* The deciduous woods toward the bottom of the hill are composed largely of *Quercus macrocarpa.*

No. 2. A swamp at the mouth of a spring one mile north of Freeport in Winneshiek County. The herbaceous growth in the foreground is composed chiefly of *Symplocarpus foetidus.* In the background is a clump of *Alnus incana* and a tree of *Fraxinus nigra.*

No. 3. Associes in Mississippi Valley near New Albin. *Nuphar advena* is foreground with *Sparganium eurycarpum* along the water's edge. Deciduous trees in the background are largely willow and soft maple trees.

No. 4. Upper Iowa River in Union Township in Winneshiek County.

PLATE I

PLATE II

No. 1. Upland tall grass prairie in western Winneshiek County.

No. 2. White Pine grove growing in shallow soils on limestone outcrop at the Lower Dam in Upper Iowa River Valley.

No. 3. Grasslands on sandy river terrace land in Upper Iowa River Valley 10 miles west of New Albin. *Bouteloua hirsuta* is the dominant species. The grass grow- in the path and lighter in color than the surrounding vegetation is *Leptoloma cognatum.* Other species present are *Bouteloua curtipendula, Monarda punctata,* and *Verbascum Thapsus.*

PLATE II

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A COLORIMETRIC METHOD FOR THE MICRODETERMINATION OF DIACETYL[1]

E. A. PRILL[2] AND B. W. HAMMER

From the Dairy Industry Section, Iowa Agricultural Experiment Station

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Diacetyl is a flavor contributant of butter and various other food products. Although there have been extensive studies of this substance and its apparent biochemical precursor, acetylmethylcarbinol, relatively few data are available on the diacetyl content alone (as distinguished from acetylmethylcarbinol plus diacetyl) of dairy products or other foods. The reason for this has been the lack of suitable analytical methods for measuring the small amounts of diacetyl which suffice to influence the flavor of a food product.

The gravimetric method for the determination of diacetyl, more usually acetylmethylcarbinol plus diacetyl, as nickel dimethylglyoximate is satisfactory when fairly large amounts are present, but owing to the slight solubility (1) of this derivative attempts to measure 1 or 2 mg. of diacetyl in 100 ml. of distillate yield low results or even fail in the qualitative detection of it. Kunze (7) recently described a micromodification of this method, but recommended colorimetric determinations for amounts less than 0.3 mg.

At the Iowa Agricultural Experiment Station a colorimetric procedure has been developed which can be applied to the determination of very small amounts of diacetyl in butter culture, cream, butter and other materials. A description of it is given herein.

REVIEW OF COLORIMETRIC METHODS

Testoni and Ciusa (16) appear to have been the first to use a colorimetric method for diacetyl. They oxidized the nickelous dimethylglyoximate and obtained a soluble red complex in which the nickel has a higher valence number.

Barnicoat (1) dissolved the precipitated nickel dimethylglyoximate in chloroform and used the resulting yellow solution for comparisons. Mohr and Wellm (10) improved this method by extracting with chloroform the nickel complex which still remained in solution as well as that which was precipitated.

Pien, Baisse and Martin reacted the diacetyl with certain ortho-diaminobenzene derivatives and made use of the yellow colors which the resulting quinoxaline derivatives exhibit in the presence of strong acid as the bases of their methods. In their first procedure (12) they used 3,4-diaminotoluene. Later (13), they used diaminobenzidine and obtained a stronger yellow color which they assumed resulted through the forma-

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tion of a bisquinoxaline derivative and which they claimed to be useful for measuring as little as 0.05 mg. of diacetyl in 10 ml. of distillate.

The Voges-Proskauer reaction, as improved by Barritt (2), although very sensitive, is not applicable to the quantitative determination of diacetyl because the color developed is not proportional to the concentration; furthermore, acetylmethylcarbinol also gives the test. Certain color reactions reported by Smith (15) might possibly be made the bases of other color tests.

Titrimetric methods for diacetyl are also possible such as that of Ruehe and Corbett (14), but it is doubtful whether these could be as sensitive and specific as a suitable colorimetric method for microdeterminations.

DEVELOPMENT OF THE METHOD

Tschugaeff and Orelkin (17, 19) developed a procedure for the colorimetric determination of very small amounts of iron by the use of dimethylglyoxime and ammonium hydroxide. Just as the converse of the procedure for the gravimetric determination of nickel with dimethylglyoxime provided the basis for the gravimetric method for diacetyl, so the converse of the procedure for iron constitutes the basis for a colorimetric method for small amounts of diacetyl. The intensely rose-red colored, water-soluble complex that is produced under the conditions of the test is an ammono-ferrous dimethylglyoximate having the probable formula

$$\text{DH-Fe-HD} \;.\; 2NH_3$$

(DH_2 indicates that dioxime and DH- indicates that the H of one of the two oxime groups has been replaced by metal). The compounds which iron as well as nickel and several other metals form with certain dioximes belong to the class of substances now often known as chelate ring compounds. These are discussed in recent reviews (3, 8).

The diacetyl is first converted into diacetyl dioxime (dimethylglyoxime). In the present procedure this is accomplished by the use of a solution containing approximately equimolar quantities of hydroxylamine hydrochloride and sodium acetate and which is therefore essentially the same as a solution containing hydroxylamine acetate and sodium chloride. Such a solution is slightly acidic, because hydroxylamine is a very weak base. Preliminary investigations (unpublished) on the formation of the dioxime show that this reagent is satisfactory under the prescribed conditions (concentration and period of heating which are indicated later) and that a neutral, alkaline, or strongly acidic solution of this reactant is less satisfactory for the present purpose.

For the subsequent color producing reactions to be satisfactory, it was found necessary to remove the excess hydroxylamine remaining after the completion of the formation of the dioxime. In the trials performed before this fact was appreciated, and in which the excess of hydroxylamine was not removed before applying the subsequent reactions, the colors obtained faded rapidly and developed a brownish tinge. The reason for this is that hydroxylamine in alkaline solution oxidizes ferrous iron to ferric iron (9). The excess of hydroxylamine can be fixed by the addition of acetone, whereby it is converted into acetoxime which does not

interfere. It was suspected, however, that the acetone also might react with the dioxime of diacetyl to produce acetoxime and the monoxime of diacetyl, thereby partly undoing that which was accomplished in the first step of the method.

When acetone was added to a test solution, in which the dioxime formation had been completed and which was slightly acid, and the solution then heated at 80° C. for 1 hour, the color which developed after the addition of the color producing reagents was somewhat less intense than that which developed in a comparison test solution which had been heated only 5 minutes after the addition of acetone. When the proper amount of dipotassium phosphate was added along with the acetone so that the resulting solution was nearly neutral (pH 6.7) it was found that the intensity of the final color obtained was not affected by the period of heating following the addition of the acetone. This indicates that acetone reacts with the dioxime in acid solution but not to a detectable extent in nearly neutral solution.

In the next step it is necessary to add some reagent to prevent the precipitation of the excess iron as iron hydroxide when ammonium hydroxide and ferrous sulfate are added to the solution containing the dioxime. Potassium sodium tartrate was found to be very effective. A small amount of the tartrate prevents the formation of a precipitate but the solution is colored intensely blue due to tartrate-soluble ferrous-ferric hydroxide (slight oxidation of the iron by air is unavoidable in alkaline solution). By using a relatively large amount of the tartrate this color is suppressed so that only a very faint green tint remains. Komàrek (6) made similar observations in connection with the development of his titrimetric method for iron. The high concentration of tartrate in the to fade because of the oxidation of the excess ferrous iron by the air.

It was found that the ferrous sulfate should be last in the sequence in which the several reagents are added. It was noted also that a relatively small amount of it was sufficient, which is fortunate because this makes it possible to so reduce the slight green color caused by the reagents that it is negligible. On the final addition of the ferrous sulfate the beautiful rose-red color appears instantly. The color attains its full intensity in a few minutes and is stable for several hours, but then begins to fade because of the oxidation of the excess ferrous iron by the air.

Some explanation of the other complexes that iron forms with dimethylglyoxime seems pertinent to the reasons why the ferrous sulfate should be added last. This reagent might be contaminated with a trace of ferric salt. Ferric iron under certain conditions also forms a complex with dimethylglyoxime (5), but if the addition of tartrate or of tartrate plus ammonium hydroxide precedes the addition of the iron salt the ferric complex does not appear to be formed. There are also other ferrous complexes. It was observed that when a very minute quantity of ammonium hydroxide is added to a solution containing the dioxime, tartrate and ferrous sulfate, the color becomes dark brown, which appears to be due to the same dark brown ferrous complex (18) that would be formed if sodium hydroxide were added in place of the ammonium hydroxide. On the subsequent addition of an excess of ammonium hydroxide the resulting color is a mixture of red and brown; the brown complex appears to be so stable when once formed that it persists even on the addition

of an excess of ammonium hydroxide. If the ammonium hydroxide were added last in the analytical procedure, a condition similar to this might be obtained in the lower part of the test tube because of the low concentration of the ammonium hydroxide that had reached the lower part of the tube by diffusion. This would be true especially if the contents of the tube were not mixed immediately.

It should be mentioned also that if certain amines are substituted for the ammonium hydroxide in the regular analytical procedure, different reddish or purplish colors are developed.

REAGENTS

Blank determinations should be performed on the chemicals used in the preparation of the reagents. The acetone and the methyl alcohol certainly should be tested since conceivably these might contain traces of diacetyl or one of its homologs. Distilled water should be used throughout.

A. Hydroxylamine acetate

Dissovle 70 g. of sodium acetate ($NaC_2H_3O_2 \cdot 3H_2O$) in water to make 200 ml. of solution. Dissolve 35 g. of hydroxylamine hydrochloride in water to make 800 ml. of solution. Prepare a day's supply of the reagent by mixing the two solutions in the proportion of 1 part of the first to 4 parts of the second by volume.

B. Acetone-dipotassium phosphate mixture

Dissolve 144 g. of anhydrous dipotassium phosphate or 190 g. of its hydrate ($K_2HPO_4 \cdot 3H_2O$) in water, add 200 ml. of pure acetone and make up to a liter with water.

C. Ammonium hydroxide

Concentrated C.P. ammonium hydroxide (sp. gr. 0.90) is used.

D. Saturated tartrate solution

As needed, dissolve 90 g. of potassium sodium tartrate (Rochelle salt) in 50 ml. of water by warming. Keep warm (about 35° C.) in order to prevent crystallization.

CD. Mixture of C and D

In treating a number of samples at a time it is more convenient to add C and D together. Just before it is needed, prepare the mixture in the proportion of 100 ml. of the warm tartrate solution to 13.5 ml. of concentrated ammounium hydroxide. Use immediately so that crystals do not separate from the solution due to cooling.

E. Ferrous sulfate

Dissolve 5 g. of ferrous sulfate ($FeSO_4 \cdot 7H_2O$) in 100 ml. of a 1 per cent solution of sulfuric acid. This solution should be discarded whenever it shows evidence of oxidation.

F. Dilution solution

Ordinarily this solution is not required, but if dilutions are found to be necessary after the color has been developed they should not be made with water but rather with a freshly prepared mixture of reagents made up in the following proportion: Distilled water—63 ml.; B—10 ml.; C—3 ml.; D—22 ml.; and E—2 ml.

G. Diacetyl standard

It is not practical to use diacetyl itself as the standard because absolutely pure diacetyl is not readily available, it is inconvenient to weigh accurately, and its solution has been reported to be unstable. Since the diacetyl in the unknown is actually converted into dimethylglyoxime in the course of the analytical procedure, there should be no objection to its use in place of diacetyl in making the standard solution, provided that the standard and the unknown solutions are given identical treatment in all other respects.

Weigh out 0.1349 g. of purified dimethylglyoxime (recrystallized from alcohol or from a large volume of water) and dissolve in 50 ml. of pure methyl alcohol. When all the crystals have dissolved add 30 ml. of reagent A (hydroxylamine acetate) and make up to a liter with water. One ml. of this solution contains 0.1349 mg. of the dioxime, which is equivalent to 0.100 mg. of diacetyl. The dilutions needed are freshly prepared each time.

The function of the hydroxylamine acetate in this solution is to repress any possible hydrolysis of the dioxime, to fix any trace of aldehyde that may possibly be in the solvent, and to inhibit any microorganisms which might otherwise grow in the solution.

DISTILLATION APPARATUS AND DISTILLATION PROCEDURE

The quantitative collection of the diacetyl from a sample in a small volume of distillate requires a special distillation apparatus and very carefully regulated distillation. After trying various types of apparatus which did not give entirely satisfactory results, the apparatus shown in figure 1 was devised and was found to be satisfactory.

It would perhaps be better if ground glass connections were used, but the present apparatus can be easily made from the usual laboratory equipment. Ordinary rubber stoppers, however, should not be employed since tests on shavings from such stoppers after being used for distillations showed the presence of detectable quantities of absorbed diacetyl. The connections which come in contact with the vapor, B_1 and B_2, may be made with synthetic rubber (neoprene). Shavings from neoprene stoppers after being used in distillations gave negative tests for diacetyl.

The inlet tube D is for the introduction of CO_2 and steam; either of the openings, E for CO_2 or F for steam, can be closed by means of a clamp on the rubber tubing connections when the other is being used. The tube D can be rotated in the stopper so that the CO_2 or the steam can be passed at will either through or above the sample in the flask. The water connections to the condenser jackets J_1 and J_2 should be so arranged that J_1 is always filled with water while J_2 can be easily filled or emptied, thus allowing the tube C to be used at will either as a reflux condenser or as a fractionating column.

In the tube C are placed some glass discs, G, about 15 in number, which are held about 20 mm. apart by means of short sections of glass rod attached to them. The lowest disc is attached to a longer section of rod (about 100 mm.), which rests on the wall of the bent section of tube C and thereby supports the column of discs. When C is used as a reflux the discs act as baffles and minimize the escape of diacetyl. When C is em-

ployed as a fractionating column it is essentially a simple form of the Young (20) column. The inner diameter of tube C may be 10 to 12 mm. and the discs should be ground so that their diameters are just slightly less than this (about 1 mm. less). Whether the discs fit properly or whether the apparatus as a whole is satisfactory can only be determined

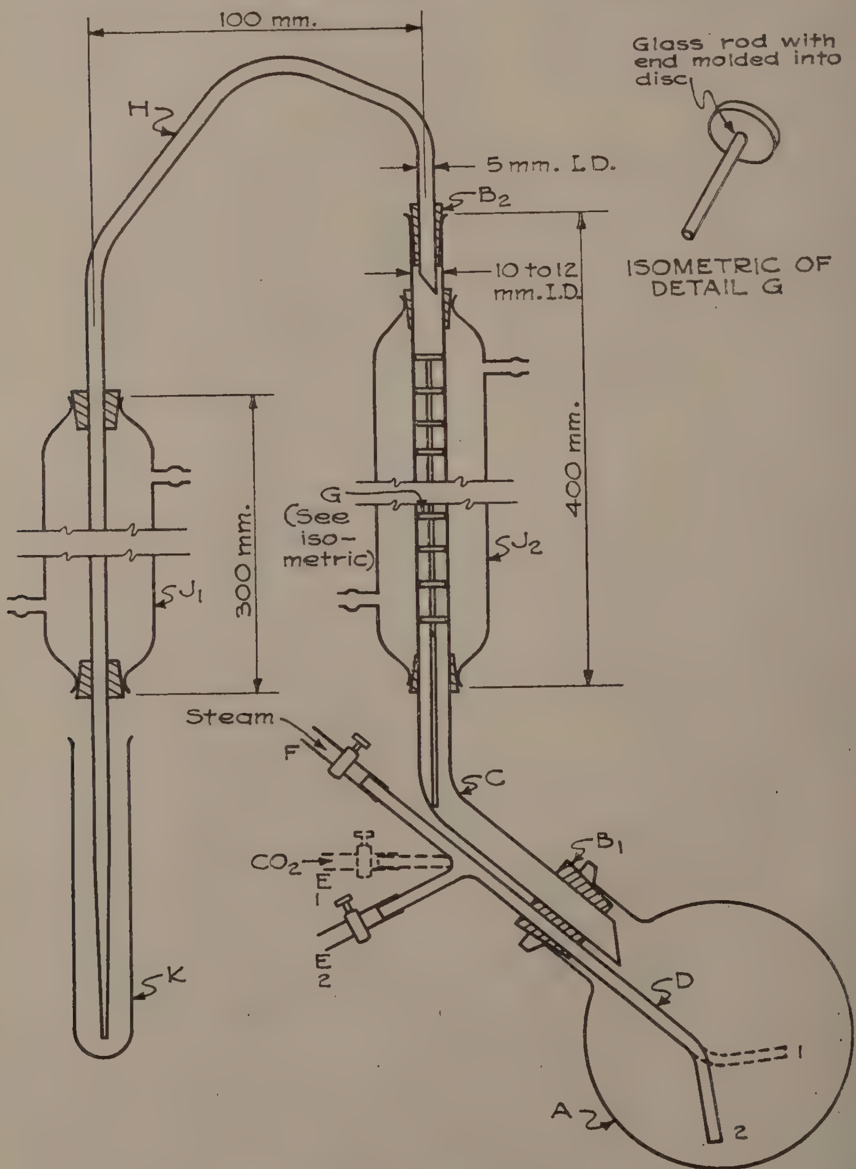

Fig. 1. Diagram of distillation apparatus.

by actual trial distillations, wherein a solution containing about 1 mg. of diacetyl is distilled in the manner described in the following paragraphs, and several successive 5 ml. portions of the distillate collected. If all the diacetyl is consistently found in the first portion of distillate, the apparatus has been constructed in the proper manner.

Before an actual distillation is performed the apparatus should be cleaned by distilling water through it. The sample is weighed into the 500 ml. round bottom flask A; 50 g. is usually a sufficient and convenient amount, but for materials low in diacetyl a 100 g. sample may be used. The sample should be slightly acidic in reaction, which is usually the case, but with materials which could possibly have a reaction of pH 7 or higher, for example, butter from neutralized cream, the reaction should be made slightly acidic by the addition of a small amount of some weak non-volatile acid, such as lactic (blank determinations on the acid should show the absence of interfering substances). An alkaline reaction is avoided because even a slight alkalinity will tend to cause the destruction of diacetyl (11, 4), especially on heating. The flask is attached to the apparatus with the inlet tube D in position 1. Water should be running through both jackets J_1 and J_2. The narrow test tube K, which is calibrated at 10 ml. and also marked at approximately 6 ml., is used as the receiving vessel. One ml. of reagent A (hydroxylamine acetate) is pipetted into the test tube which is held so that the constricted end of tube H extends to the bottom of the absorbing liquid.

A slow stream of CO_2 is passed over the sample and through the apparatus for 5 minutes. Then with D still in position 1 the CO_2 inlet is closed and the steam is slowly admitted until the vapor starts to condense in the reflux C, whereupon D is carefully turned to position 2. The steam should be so regulated that the CO_2 which it displaces bubbles through the liquid in K at a slow rate.

There are reasons for employing these preliminary operations. If the air is not previously driven from the apparatus with CO_2, the atmospheric oxidation of the acetylmethylcarbinol (which is usually present in larger amounts than the diacetyl) to diacetyl on heating may lead to results which are too high. On introducing steam above the surface of the sample, the CO_2 and any remaining air are displaced by steam before the sample is greatly heated. It was observed also that when CO_2 was bubbled through a liquid sample or when the distillation was performed without the preliminary slow displacement of the CO_2 by steam under a reflux, a considerable fraction of the diacetyl was carried over with the CO_2 before any liquid actually distilled over and a certain percentage of this diacetyl was lost by being carried away with the bubbles of gas passing through the absorbing solution in K. With the procedure outlined very little of the diacetyl is prematurely carried over with the CO_2 and if several per cent of this small amount escapes absorption the loss is negligible. Refluxing is also often advantageous because after the sample has been refluxed until the initial frothing has subsided the distillation can proceed more smoothly.

When the rate of bubbling through the liquid in K indicates that the CO_2 has been displaced from the flask, the water is let out of J_2. After the column C has become warm the steam pressure should be so regulated that the distillation proceeds at a uniformly slow rate. If the rate of distillation is too rapid the column becomes flooded and its efficiency is de-

stroyed; also the required volume of distillate is soon collected but all of the diacetyl is not contained therein. The necessity for slow distillation cannot be overemphasized. If the volume of liquid in the test tube increases at a barely perceptible rate and the tube H is hot below the point where it starts to bend downward into the jacket J_1, the distillation is progressing effectively. This slow rate of distillation is maintained until the volume of liquid in the test tube K is between 6 and 6.2 ml. During the collection of the last 1 ml. the rate of distillation is increased slightly, the test tube K is lowered, and the distillate allowed to drip into it. The tube of distillate can be stoppered and kept until it is convenient to develop and compare the colors of a series of samples. The distillation proper should take approximately 30 minutes. One analyst can take care of several distillations at a time.

PROCEDURE FOR THE DEVELOPMENT AND COMPARISON OF THE COLORS

The tubes of distillate which have been collected each contain 1 ml. of reagent A (hydroxylamine acetate) and 5 to 5.2 ml. of distillate. The stoppers of the tubes are loosened and the tubes heated in a water bath at 85° C. for 1 hour in order to complete the formation of the dioxime.

The tubes are then removed from the water bath and while they are still warm 1 ml. of reagent B (acetone-dipotassium phosphate) is added to each and allowed to react for 5 minues. This fixes the excess of hydroxylamine. The tubes are cooled and may be kept at this stage if desired.

To each of the tubes are next added 0.3 ml. of reagent C (ammonium hydroxide) and 2.2 ml. of reagent D (saturated tartrate solution) and the contents of each tube mixed; or, if more convenient, 2.5 ml. of the mixed reagent CD is added in place of C and D separately.

Each tube is then treated with 0.2 ml. of reagent E (ferrous sulfate) and the contents of the tube mixed *immediately* by inverting. The volumes are brought to 10 ml. by the addition of distilled water and again mixed. Although the color develops very rapidly, about 15 minutes should be allowed for it to attain its full intensity. The colors should be compared within 2 hours.

The color standards are made by adding the desired amount of solution G (diacetyl standard) or one of its dilutions to 1 ml. of reagent A (hydroxylamine acetate) and bringing the volume up to 6 ml. with water. These standards are then treated in exactly the manner described for the unknowns. For color standards ranging from 0 to 0.010 mg. of diacetyl, a series of tubes varying by 0.001 mg. should be prepared for direct visual comparisons. For the higher concentrations to be used with a colorimeter a series increasing in appropriate amounts, for example, 0.010, 0.015, 0.025, 0.040, 0.060, 0.100, 0.150 mg., etc., is prepared; the comparisons are more easily made with a blue filter in the colorimeter.

If it is desirable to collect a different volume of distillate and have a final volume other than 10 ml., the quantities of all the reagents used for the unknowns and for the color standards, with the obvious exception of the solution G (diacetyl standard), should be changed proportionately.

SENSITIVITY, ACCURACY AND SPECIFICITY

With the method described it is possible to detect a difference between 0.001 mg. and no diacetyl in 5 ml. of water. Throughout the range

of diacetyl contents studied, the color appears to be proportional to the concentration of diacetyl, and in the range that can be conveniently measured with a colorimeter, which varies from about 0.01 to 0.5 mg. of diacetyl in the final volume of 10 ml., the proportionality is clearly evident.

Table 1 presents a comparison of the results obtained by the colorimetric and the gravimetric methods; the values were selected as representative of the many comparisons made. Each unknown solution of 200 ml. was divided, using 1 per cent for the colorimetric and the remainder for the gravimetric determination. The data show a good agreement in the results of the two methods, the differences ranging from 0.6 to 4.5 per cent.

Practically quantitative recoveries were obtained when 0.2 mg. diacetyl was added to 50 ml. of fresh skim milk, and the milk acidified with 1 ml. of 85 per cent lactic acid and distilled.

It would be expected that certain volatile homologs of diacetyl would also produce colors with this new procedure as well as with the other colorimetric procedures for diacetyl to which references have been made in this paper. Glyoxal, methylglyoxal and acetylpropionyl were tested according to the present procedure and all were found to produce intense red colors. In addition, pyruvic acid, which in a sense is a 1,2-dicarbonyl compound also, was found to produce a yellow color with the reagents used. Since these other substances ordinarily would not be expected in dairy products, the method is applicable to the analysis of these products for diacetyl.

Pure acetylmethylcarbinol does not produce a color with the reagents used. The method can, however, be applied to the determination of this compound by first oxidizing to diacetyl.

SUMMARY

A sensitive colorimetric method has been developed which can be applied to the determination of very small amounts of diacetyl in dairy products and perhaps other materials.

The method is based upon the formation of the intensely colored ammono-ferrous dimethylglyoximate. The diacetyl is first converted into dimethylglyoxime; the excess of hydroxylamine is fixed with acetone in phosphate buffered solution; then ammonium hydroxide, a large amount of tartrate and, finally, a small amount of ferrous sulfate are added. The rose-red color develops very rapidly.

An apparatus and a procedure for the distillation of the diacetyl from the sample are described.

TABLE 1. *Comparison of the results obtained by the colorimetric and by the gravimetric methods*

Colorimetric p. p. m.	Gravimetric p. p. m.	Difference Percentage	Colorimetric p. p. m.	Gravimetric p. p. m.	Difference Percentage
171	170	+ 0.6	177	173	+ 2.2
207	204	+ 1.4	173	170	+ 1.7
107	108	− 1.0	127	130	− 2.4
160	162	− 1.2	45	43	+ 4.5
154	148	+ 4.0	68	66	+ 3.0

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CYTOLOGICAL AND MORPHOLOGICAL FEATURES ASSOCIATED WITH IMPOTENCY OF POLLEN OF THE WINESAP APPLE

JOHN N. MARTIN

From the Department of Botany, Iowa State College

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It is well known that the pollen of the Winesap and its seedlings is characterized by a high percentage of impotency. Shoemaker (11) states that the varieties of the Winesap group are noted for self-, inter-, and cross-sterility and are further characterized by a low percentage of pollen germination. Knowlton (6) states that the varieties, Stayman, Winesap, and Mammoth Black Twig are not only self-sterile but of little value as pollenizers for other varieties because of their impotent pollen. Beaumont and Knight (1), who also report that the Winesaps are notoriously poor pollen parents, show by comparative tests of germination and of tube growth on artificial media that Winesap pollen is much inferior in both germination and tube growth to the better pollen producers. such as Delicious. While the average germination of Winesap pollen was 65 per cent and tube growth 1.89 mm., the germination of Delicious pollen was 88 per cent and tube growth 2.81 mm. In comparative germination tests of the pollen of Winesap, Stayman Winesap, Paragon and Delicious, Shoemaker (11) obtained 65.5, 36.9, 28.3, and 88.5 per cent, respectively. Shoemaker further reports that in cross pollination experiments the set of fruit when Delicious pollen was used was double that obtained with Winesap pollen.

Cytological studies have shown that cultivated apples, with few exceptions, if any, fall into two groups, diploids with 34 chromosomes and triploids with 51 chromosomes. It has also been well established by the investigations of Shoemaker (11), Kobel (7), Crane and Lawrence (2), Miedzyrzecki (8), Roscoe (10), Heilborn (5), and Edgecombe (4), and others, that irregularities in meiosis and abortion of pollen characterize triploidy in apples. Crane and Lawrence (2) in their studies found the range of good pollen 4 to 27 per cent in triploids and 50 to 70 per cent in diploids.

That the pollen impotency of the Winesap is traceable to a triploid condition is naturally expected, but Nebel (9) reports that the Winesap is a diploid with 34 chromosomes. If Nebel is correct then the pollen impotency in the Winesap must be due to special pollen lethals or factors other than triploidy.

MATERIALS AND METHODS

These investigations were made intermittently, in 1924, 1930, and 1936. Most of the material was obtained from the Horticultural orchards of Iowa State College at Ames, some from the College orchard at Charles City, Iowa, and some from private orchards in the state. Some of the material was from young and some from old trees. No significant differences were observed with respect to time, place collected or age of trees from which it was collected.

The material for the meiotic studies was freed of bud scales, a few anthers examined in aceto-carmine or methylene blue to determine stage of development, and if in desired stage the remainder of the flower bud was killed and fixed in Bouin's solution. In following the progress of pollen abortion and associated changes in other tissues of the anther the flowers were sufficiently developed so that only the anthers were carried through the processing. The material was all processed by the paraffin method and sectioned six to nine microns in thickness. Most of the sections were stained with iron-alum haematoxylin but some were stained with safranin and fast green.

MATURE ANTHERS

The pollen output of Winesap anthers is far below the normal, probably considerably less than half of normal anthers. The mature pollen varies from none in many of the anthers to a normal amount in an occasional anther.

According to evidence presented later in the discussion, both the impotency and low production of pollen in the Winesap are apparently associated with a reversal in the physiological relationship between the pollen and tapetal cells. In normal anthers the tapetal cells are supposedly digested and utilized by the developing pollen (fig. 2). Furthermore, the disappearance of the tapetal cells in normal anthers adds considerable space to the pollen cavities and is an important means of accommodating the space of the locule to the growing demand for space by the developing pollen grains. In most of the Winesap anthers this physiological relationship is reversed, and the tapetal cells, instead of being digested by the growing pollen, encroach upon the pollen, limit it in space, and apparently digest a variable number of the pollen grains.

Associated with the reversal in the physiological relationship between tapetal cells and pollen, there is commonly an abnormal development of some of the cells of the inner portion of the walls of the pollen sacs. In the normal development of pollen sacs there is very little thickening of the pollen sac walls during growth and maturing of the pollen. Cell divisions are mostly, if not entirely, anticlinal and result in the enlargement of the locular space and not in the thickening of the pollen sac walls. In the Winesap the wall cells of the inner side of the locules of the abnormal anthers commonly undergo periclinal divisions and thus form vegetative tissue that intrudes into the locules. In anthers where there is little or no pollen matured the locules are often entirely filled with the protruding wall tissue. This abnormal development of the wall cells of the pollen sacs is subsequent to the excessive development of the tapetal cells which break down and disappear as they are encroached upon by the wall cells.

The abnormal developmental history within the Winesap anthers is not accompanied by any very marked deviation from normal anthers in either size or form (fig. 1). In the anthers of the Winesap the ratio of breadth to thickness is apparently greater than in the anthers of most apple varieties but there is no noticeable collapsing, even in anthers where there is complete destruction of pollen. The vegetative wall tissues intruding into the locules and filling the space normally occupied by pollen prevents the collapsing of pollen sacs and any marked deviation of the anthers from normal appearance in size and shape.

PRE-MEIOTIC AND MEIOTIC STAGES

There was no suggestion of abnormalities in either the pre-meiotic or meiotic stages of anther development. The various steps in the development of the chromosomes were normal for apples. Likewise at these stages of anther development the tapetal cells and wall cells of the pollen sacs were normal. The tapetal cells were heavily loaded with food, which is a normal condition, were normal in size and displayed no aggression toward the pollen mother cells. In all stages up to and somewhat beyond the tetrad stage there was no apparent aggression of the tapetal cells. The mother cells had ample and approximately normal amount of space for development and formation of tetrads (figs. 4, 6 and 7).

In meiosis there were some multiple associations of chromosomes, a feature well known in apples. Excepting a very occasional lagging of a chromosome, both the reduction and homotypic divisions of meiosis were perfectly normal (fig. 4). The number of chromosomes estimated from a number of counts made in diakinesis, meta- and anaphase is 34, which is in agreement with Nebel's report. Following meiosis the tetrads increase in size, form their walls and separate, apparently in the normal way. The first indication of pollen abortion does not appear until the pollen grains have attained the globular form, approximately full size, and have their walls fairly well developed. Then it is that the protoplasm of many, if not most, of the pollen grains is noticeably scanty, vacuolate, and lacking in density.

THE ENCROACHMENT OF TAPETAL AND WALL CELLS AND ABORTION OF POLLEN

When pollen showed decided evidence of a disturbance in its development by the scantiness, lack of density, vacuolated condition and disorganization of protoplasm, the tapetal cells had noticeably elongated and closed in on the locular space, and had contacted many of the outlying pollen grains (figs. 5 and 8). In many locules the pollen grains were crowded into a compact mass in the central area of the locule (fig. 8).

The tapetal cells showed no evidence of undergoing the digestion that occurs during normal development of pollen grains. Their protoplasm had greatly increased and had lost none of its density. The evidence of the pollen being digested was generally more noticeable in the pollen grains nearest the tapetal cells but those in the center of the locule were not far behind in showing a loss of cell contents. Accompanying the loss of cell contents there was a shriveling of pollen and commonly complete disorganization, only a small dark remnant being left. In extreme cases the encroachment was continued until the locule was obliterated and pollen all aborted (figs. 3, 10 and 11), but generally some pollen grains escaped destruction, although the protoplasm of many of these was abnormal in appearance. Although apparently full size, their protoplasm was vacuolate and lacked normal density. This can account for the impotency of much of the Winesap pollen as found in germination tests and pollination experiments.

After the tapetal cells had run their course, their protoplasm usually lost much of its density, and commonly they disorganized and shrank away, the space occupied by them being appropriated by the ingrowing

wall cells of the locule (figs. 10 and 11). Possibly the wall cells digest the tapetal cells and make use of them in their extra development.

A study of dehiscense was not made, but with few exceptions, as in figure 11, the mechanism for dehiscence was developed and anthers with almost no pollen were found in the process of dehiscing as shown in figure 9.

DISCUSSION

Pollen impotency to the extent that some of the pollen grains are shriveled and non-functional is quite general in diploid apple varieties where pollen impotency can not be attributed to irregularities in meiosis found in triploids. In diploids as much as 20 per cent dead and shriveled pollen is probably not uncommon and a much higher percentage may occur in some varieties. Heilborn (5) explains pollen abortion in diploid apples by assuming recessive pollen lethals, and that the chromosome constitution of the apple is according to that proposed by Darlington and Moffett (3), namely, that the basic number of chromosomes is seven and that diploid apples, although behaving as diploids, are in their fundamental chromosome constitution polyploids. They are allotetraploids supposedly formed by inter-specific crosses. That the basic chromosome number is seven is suggested by the fact that through multiple associations all the chromosomes of a complement are sometimes included in seven groups in meiosis. According to Darlington and Moffett the seven groups consist of three sexivalents and four quadrivalents which may be represented in the haploid condition by AAA, BBB, CCC, DD, EE, FF, and GG. Now if one chromosome of each of the homologues of one or more of the groups has pollen lethal factors the varied assortment of chromosomes in meiosis should give some pollen homozygous for recessive lethals and these would abort. The amount of abortion would vary with the number of chromosomes of a group having lethal factors and with the number of groups having chromosomes with lethal factors. Let us represent the chromosomes carrying recessive pollen lethal factors by small letters and suppose a certain apple tree has the diploid chromosome constitution AaAaAa, BBBBBB, CCCCCC, DDDD, EEEE, FFFF, and GGGG. Approximately one-eighth of the pollen produced by this tree should receive the three lethal factors aaa and be sterile. If in addition to the lethal factors in the A group, the B group has the constitution BbBbBb, then approximately one-fourth of the pollen should be sterile. It is easily seen how various degrees of sterility can result from such a chromosome constitution with a variable number of chromosomes bearing recessive pollen lethal factors.

In the Winesap, pollen sterility is not merely a matter of the pollen's failing to reach normal maturity with no apparent cause outside of the protoplasm of the pollen; but there is the accompanying abnormal behavior of the tapetal cells and to some extent of the wall cells of the pollen sacs, that strongly suggests a reversal of the physiological relationship between the pollen and surrounding cells, especially the tapetal cells, which results in the larder's getting control of the household and eating up the masters.

The specific relationship between the developing pollen and the tapetal cells in normal anthers is not known. The general condition is that there

is only a mere trace of the tapetal tissue at the time of the anther's maturity. It is supposedly consumed by the developing pollen. Possibly the tapetal tissue inherently reaches a stage of maturity earlier and gives itself over, figuratively speaking, to be consumed by the developing pollen; or it may be that the developing pollen has greater imbibing power and more efficient digestive enzymes and simply subdues and consumes the tapetal tissue. The latter view has at least three things in its favor in explaining the situation in the Winesap. Such a view would explain the over-development of the tapetal tissue and its encroachment upon and apparent digestion of the pollen on the basis that the developing pollen is low in vigor and consequently is overpowered by the tapetal tissue; or that the tapetal tissue is extraordinarily vigorous, and therefore superior to the pollen in absorbing power; or that the disparity between the pollen and tapetal tissue is caused by a lack of vigor in one and excess of vigor in the other. That there is a competitive relationship between pollen and tapetal tissue is supported by the fact that in anthers with excessive tapetal tissue development, commonly a few of the tapetal cells behave in the normal way, apparently undergoing digestion as is usual in the normal development of anthers. Another feature favoring the competitive relationship is the wide variation in the disturbance in the different anthers, ranging from total to almost no abortion of pollen. In diploid varieties where pollen abortion supposedly results from recessive pollen lethals some variation in abortion of pollen in different anthers should be expected, but not such wide variation as occurs in the Winesap.

Physiological as well as other features of organisms are fundamentally gene influences. The ability of pollen grains and tapetal cells to digest and absorb materials surrounding them doubtless is traceable to gene influences. As a result of the supposedly hybrid constitution of the apple, the abnormal physiological relationship between the tapetal cells and pollen of the Winesap very likely is caused by an unusual gene constitution. It may be that in the particular gene constitution of the Winesap the double number of genes for ability of the tapetal cells to absorb and digest gives them an advantage over pollen with the haploid number of genes. Whatever the cause may be, it no doubt is as inherent as the abortion by causes apparently entirely within the pollen.

If the relationship of absorptive ability between pollen and tapetal tissue is not far from being balanced, then competition between flowers, between anthers of the same flower for water, carbohydrates and minerals, and differences in temperature to which different anthers are exposed during their development may affect the balance and account for the wide variation in the disturbance of different anthers.

SUMMARY

1. Pollen abortion in the Winesap is associated with abnormal development of tapetal tissue and pollen sac wall cells.

2. The tapetal tissue instead of diminishing and practically disappearing during the development of the pollen and the normal maturing of the anther, continues its development to the extent that it crowds in upon the pollen. In extreme cases the tapetal tissue practically fills the locule, leaving very little room for pollen development.

3. As the pollen is encroached upon by the tapetal tissue, it shows evidence of abortion in lack of density and in the vacuolated condition of protoplasm and finally in shriveling and disorganization.

4. It is suggested that pollen abortion in the Winesap is caused by a reversal of physiological relationships in which the tapetal cells digest the pollen.

PLATE I

Fig. 1. Mature stamens of Winesap showing general appearance of anthers.

Fig. 2. Lengthwise section through an anther with normal development of pollen and digestion and disappearance of tapetal cells.

Fig. 3. Lengthwise section through abnormal anther of Winesap in which both pollen and tapetal tissue have been largely replaced by ingrowing wall tissue. Note cross-sectional view in figures 9 and 10.

Fig. 4. Lengthwise section through locule of Winesap showing meiosis proceeding normally.

Fig. 5. Cross section of anther showing tapetal cells encroaching upon the pollen which is already showing disorganization.

Fig. 6. Cross section of locule showing mother cells normal in appearance.

Fig. 7. Tetrad stage of pollen development with no abnormalities in development yet discernible.

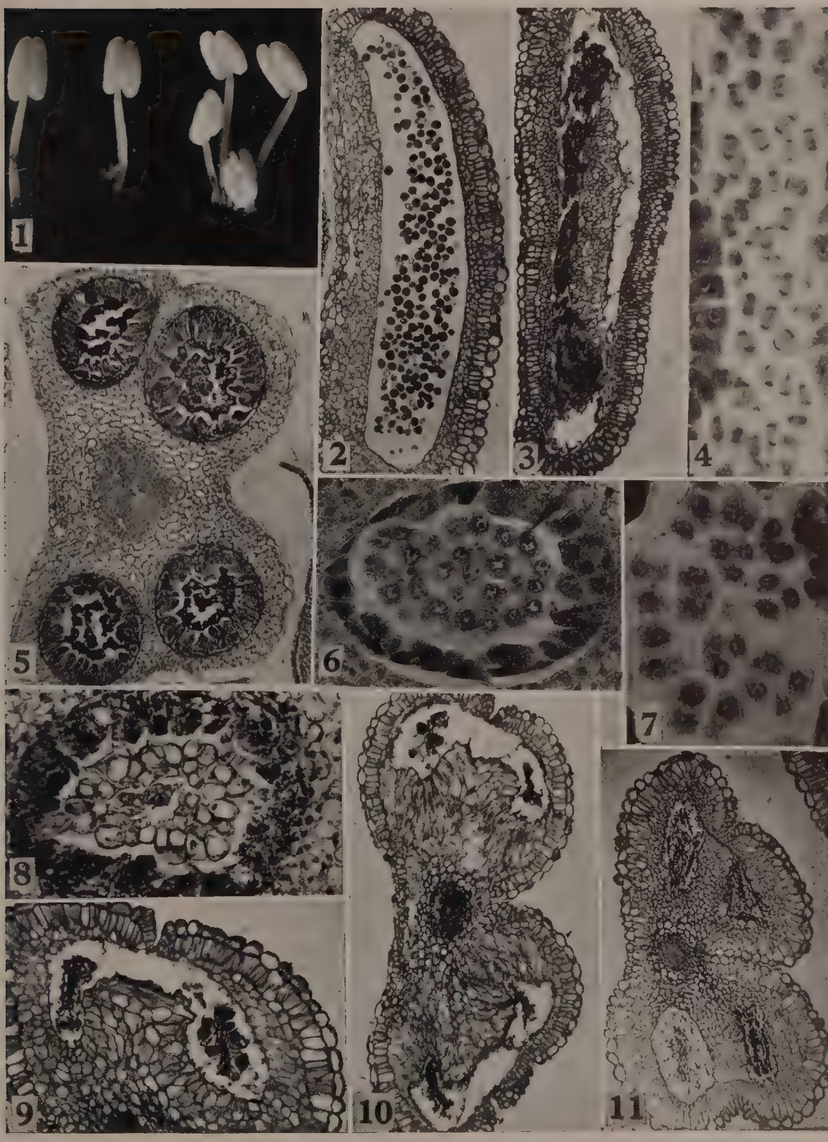
Fig. 8. Cross section of a locule at the stage in which pollen is being crowded by tapetal tissue. Pollen is compacted, vacuolated, and nuclei are disorganized.

Fig. 9. Cross section of locule in which pollen and tapetal tissue have been replaced by wall tissue. Note that wall cells have undergone the modifications that constitute the mechanism for dehiscence.

Fig. 10. Similar to Fig. 9, but showing both locules in which the ingrowing of the wall tissue and absorption of tapetal tissue and pollen are extreme although a common occurrence in Winesap anthers.

Fig. 11. Cross section of an anther in which development of wall tissue has occurred on all sides of the locules and development of the dehiscing mechanism has been omitted. In lower left locule the tapetal cells remain as elongated vacuolate cells almost completely filling the locule.

PLATE I



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THE INFLUENCE OF THE RATION ON MORTALITY FROM CAECAL COCCIDIOSIS IN CHICKS[1]

ELERY R. BECKER AND PHILIP C. WATERS

From the Department of Zoology and Entomology, Iowa State College

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That diet may be an important factor in mortality from caecal coccidiosis in chicks was brought out in a recent note by the senior author (1937). Since it was promised that a more detailed account would appear later, and since other experiments along similar lines have been carried out, a further account of the progress of the work should now be published.

The method employed for attacking the coccidiosis problem through diet is unique. It is based on the hypothesis that certain feeding stuffs in the ration should favor severity more than others. In the case of *Eimeria nieschulzi* infection of the white rat, it has been demonstrated repeatedly that the quantitative character of the infection, as indicated by oocyst counts, can be controlled to a considerable extent through manipulating the formula of the ration (Becker and Derbyshire, 1937). It was hoped that similar study could be made of the number of oocysts discharged by chicks on different diets, but plugging of the caeca shortly after oocyst elimination commenced precluded attack on the problem through enumeration of oocysts. Consequently, mortality has been used as the criterion of the effect of the ration on the course of the infection. Whether the incidence of death in flocks of chicks on different diets and experimentally infected with *Eimeria tenella* is actually an index to the parasite population that has been built up in the birds is still an undetermined point, but it is clearly shown in the present investigation that one ration may predispose the hosts to fatal ravages of the parasite more than another.

The problem is really a dual one, for eventually a ration that would benefit the poultryman in reducing caecal coccidiosis in his growing flock would have to demonstrate satisfactory growth-promoting properties. A modicum of success has been attained in the direction of reducing severity of infection and maintaining growth simultaneously, but the real contribution in this paper is to point out some of the fundamental problems and difficulties involved rather than to present the poultry world with a ration that meets its requirements.

Chicks employed for experimental hosts were all White Leghorns obtained from local hatcheries. They were represented to us as having come from blood-tested, pullorum-free stock. An inquiry into the practises of the hatcherymen from whom were purchased the chicks used in the last four experiments brought out that his flocks are closely tested by a competent veterinarian known to the writer. Moreover, the Veterinary

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Investigations staff at the Iowa State College has a high regard for the health and vigor of his chicks. Autopsies of chicks that died during and previous to experimental infection with coccidia have never revealed any definite indication of pullorum infection. When the writer (E. R. B.) was in doubt he showed the carcass to veterinarians for their opinion. In one suspicious case with an unabsorbed yolk sack, a culture of the liver and other organs was made, but gave negative results. Furthermore, fatalities caused by pullorum infection usually occur between the fifth and tenth days of life, while our chicks were much older at the time of inoculation with coccidia. In addition, deaths occurring after inoculation on the fifth sixth, seventh and eighth day are quite characteristic of *Eimeria tenella*.

The microorganism, *Eimeria tenella*, was isolated from a mixed "culture" of fowl coccidia by the method of culturing scrapings from the washed caecal wall. *E. neactrix* or other forms have not been present in our culture. Three per cent potassium dichromate solution was used for a culture medium.

A particular commercial ration was selected for the control in the first three experiments because (1) it was widely used in this locality, (2) chicks died freely from coccidiosis when raised on it, and (3) the manufacturer was willing to furnish the formula. It contained the following ingredients in parts by weight: ground yellow corn, 40; hulled oats, 5; wheat middlings (gray shorts), 30; wheat bran, 5; meat and bone meal, 5; dried skim milk, 5; alfalfa meal, 13 per cent protein, 4; linseed oil meal, 1; fine oyster shell, 2; charcoal, 1; steamed bone meal, 1; salt, 0.5; cod liver oil, 1. The parts add up to 100.5. Chicks were kept on this mash from the first day of life until test feeding was started. Afterwards the control ration was mixed in the laboratory, linseed oil meal was omitted, and the salt content raised to 1 per cent. This practise insured constancy of the mixture and made the parts add up to 100. On the basis of declarations appearing on sacks in which the components of the mash were marketed the total protein content was about 15 per cent.

At the start of the experimental feeding period the chicks were divided into the desired number of lots by drawing twos and threes. They were weighed individually except where otherwise stated, and confined in battery brooders with half-inch meshed hardware-cloth bottoms. Food and water containers were located outside the cages so that the birds had to eat and drink through spaces between wires. Such arrangements prevented chicks from ingesting their droppings accidentally or otherwise. No food was given the birds except the mash in the hoppers. Repeated fecal examinations insured freedom from previous immunizing infection.

After the birds had been kept on the special diets for desired lengths of time they were inoculated directly into the crop with the numbers of oocysts that previous experience had shown to kill most of the birds on the control diet. A soft rubber catheter was found to be a safe instrument for the inoculation. In each experiment the test and experimental series received the same calculated number of oocysts per bird.

Deaths occurred on the fifth, sixth, seventh and eighth days, although in other experiments death has been observed to occur after the eighth day. Dead birds were autopsied. Hemorrhagic caeca and merozoites in the caecal content were considered presumptive evidence of the lethal effects of the parasite.

EXPERIMENT 1

The test ration in this experiment was not suitable for purposes of the poultry feeder; nevertheless, it was responsible for the first striking success in curbing mortality by manipulating the diet.

Thirteen 14-day-old chicks were weighed in at a mean of 82 gm. They were put onto the following (test) ration described in parts by weight: yellow corn meal, 25; meat and bone meal, 6; soy bean oil meal, expeller processed, 22.5; ground hulled oats, 20; ground whole wheat, 10; wheat bran, 5; ground oyster shell, 1; salt, 1; fine charcoal, 1; beet sugar, 6.5; cod liver oil, 2. The calculated protein content is 20 per cent.

Eighteen control chicks had a mean weight of 83 gm. After 9 days on the rations each chick in the two lots received an estimated 50,000 sporulated oocysts of *Eimeria tenella* from a new culture that had been thoroughly washed of the dichromate solution medium. When inoculated the test chicks showed a mean weight of 121 gm. and the controls, 142 gm. Four days later, that is, the day before deaths began to occur, the mean weights were 141 gm. and 174 gm., respectively. Six chicks were found dead in the control brooder on the fifth day and three more on the sixth day. One chick in the test brooder succumbed on the seventh day. On the seventh day the survivors in both brooders were put on the commercial ration again and observed for 10 days more without further mortality or serious illness.

EXPERIMENT 2

The test ration used in this experiment represents an attempt to improve the previous one in respect to its growth-promoting qualities. Its formula is as follows (parts by weight): yellow corn meal, 32; ground hulled oats, 20; soy bean oil meal, expeller processed, 16; fish meal, 65 per cent protein, 4; meat and bone meal, 4; wheat bran, 5; wheat flour middlings (gray shorts), 10; fine oyster shell, 2; alfalfa meal, 13 per cent protein, 2; commercial casein, 1; salt, 1; fine charcoal, 1; cod liver oil, 2. The calculated protein content is almost 21 per cent.

The chicks were kept on the commercial ration for the first 8 days of life. The 24 controls had a mean weight of 54 gm. the day after the lot was divided and the 25 test chicks, 55 gm. One of the latter group died before it was inoculated.

After a 2-weeks feeding period each bird was inoculated with a calculated 60,000 sporulated oocysts from a washed culture. Four days later, just before the onset of serious illness, the controls were found to have gained a mean of 101 gm. since the date of the previous weighing. The test chicks had gained on the average 120 gm. each. Four of the latter, however, were showing definite symptoms of nutritional paralysis of the type in which the toes turn inwards (cf. Norris *et al.*, 1931, and Record *et al.*, 1934). Fourteen of the controls died on the fifth day of the infection, 5 on the sixth day, making 19 deaths in the lot of 24 birds. The only fatality in the test group occurred on the fifth day. The findings in the caeca at autopsy confirmed the belief that all 20 chicks had died of coccidiosis.

It is of special interest that none of the four birds showing symptoms of paralysis died; in fact, they appeared to show peculiarly abnormal resistance to the infection with *E. tenella*. Be this as it may, the birds were

on their feet again within two days after they were permitted to resume the commercial ration.

The other survivors in both lots were permitted to continue on their respective rations until the thirteenth day after inoculation. For another week both lots ate the commercial ration. There were no casualties after the sixth day of the infection.

EXPERIMENT 3

The ration employed in the test lot of this series was designed with the hope that it would provide good host-growth, prevent the type of paralysis that appeared in the previous experiment, and at the same time confer a degree of coccidiosis-resistance upon the host. It has been demonstrated by Bethke, Record, and Kennard (1931) that dried buttermilk and dried skim milk would prevent the paralysis. Accordingly, a small amount of dried skim milk was included in the ration along with a greater variety of grain protein and more alfalfa meal. The following formula was the result (parts by weight): yellow corn meal, 40; ground hulled oats, 10; finely ground whole oats, 5.5; ground whole barley, 5; ground whole wheat, 5; wheat bran, 5; wheat middlings (gray shorts), 5; soy bean oil meal, 10.5; meat and bone meal, 4; fish meal, 65 per cent protein, 2; fine oyster shell, 2; salt, 1; dried skim milk, 2; alfalfa meal, 13 per cent protein, 3; cod liver oil, 1. The parts add up to 101. Unfortunately, through an oversight cod liver oil was ommitted from the formula in the previously published note. The calculated protein content is about 17.5 per cent.

When the lot of chicks was 14 days old it was divided into three groups: No. 1 received the test ration, No. 2 the control ration, No. 3 the control ration until the date of inoculation and after that the test ration. The mean weights, respectively, were 57 gm., 61 gm., and 58 gm. On the fourteenth day of the feeding period each chick was inoculated with 80,000 sporulated oocysts. On the seventeenth day No. 1 showed a mean gain of 90 gm. over the previous weight; No. 2, 86 gm.; No. 3, 88 gm. No. 1 lost on the fifth, sixth, seventh, and eighth days a total of 6 out of its 36 chicks. No. 2, the control, lost 17 out of 28. The Chi-square test gives a significant value for the difference in mortality in the two groups. The third group had 5 fatalities out of 10 chicks. It seems that the test ration was not protective when the chicks were put on it the day they were infected.

Death owing to caecal coccidiosis was confirmed by autopsy.

EXPERIMENT 4

Three rations were fed in this experiment, all of them different from the commercial ration and control in the previous three experiments. Their composition will be found in table 1.

The commercial ration was fed for the first 14 days of life. On the fifteenth day the chicks were divided into 3 lots and put on the different rations. Since other duties interfered, weights were not recorded. After 2 weeks on the special diets the chicks were infected with 125,000 oocysts each.

Ration 1 is characterized by its low dried milk content. Five out of 23 chicks receiving it succumbed to caecal coccidiosis. Strangely, no paralysis developed in this group. Ration 2 contained 6 parts of dried skim milk.

There were 10 deaths in the group of 21 that ate it. Ration 3 was made up with 6 parts of "lacto-buttermilk," a buttermilk made by culture methods and having a sour taste. There were 12 fatalities in the group of 21 chicks kept on this ration. Post mortem examinations showed the typical lesions of severe caecal coccidiosis.

The difference in mortality between the chicks on ration 1 and ration 2 was not quite statistically significant, but that between the groups on ration 1 and ration 3 was barely so.

TABLE 1. *Construction of rations used in experiment 4*

Materials	Pctg. Protein	Ration 1	Ration 2	Ration 3
Yellow corn meal	9.4	35	35	35
Meat scraps	50.0	3	3	3
Fish meal	65.0	3	3	3
Soy bean oil meal (expeller process)	41.0	10	3	3
Ground hulled oats	16.2	11	11	11
Ground whole oats	12.0	15	17	17
Wheat bran	14.5	5	5	5
Wheat middlings (gray shorts)	16.0	10	10	10
Alfalfa meal (13 per cent protein)	13.0	3	3	3
Charcoal, fine	....	1	1	1
Oyster shell, fine	....	2	2	2
Salt	....	1	1	1
Dried buttermilk (lacto-)	33.8	....	....	6
Dried skim milk	34.8	1	6	....
Cod liver oil (Nyal)	....	2	2	2
Total parts by weight		102	102	102
Percentage protein (calculated)		17.5	16.6	16.5

EXPERIMENT 5

The purpose of this experiment was to test further the effect of dried buttermilk in the ration and to obtain some idea of the independent effect of wheat middlings. The 3 rations are outlined in table 2. It is to be noted that rations 2 and 3 are well-balanced; that ration 2 is characterized by its content of buttermilk powder and wheat middlings at 10 per cent levels; that ration 3 contains buttermilk powder at the 10 per cent level and wheat middlings at the 30 per cent level; and that ration 1 more than makes up for its lack of dried milk in the 13 per cent soy bean oil meal content. Ration 1 is undoubtedly markedly limited in vitamin G. The failure of paralysis to develop in the group on ration 1 is unexplainable at present. The fish meal was prepared by drying canned ATCO, marketed as food for poultry, at 70°C. and grinding in a coffee mill.

The entire lot of chicks was fed ration 3 for the first week of life. Then each bird was weighed, numbered, and assigned to one of three brooders. The lot put on ration 1 had a mean weight of 55 gm., that on ration 2 a mean weight of 59 gm., and that on ration 3 a mean weight of 57 gm. After two weeks on the rations each chick was inoculated with 100,000 sporulated oocysts of *Eimeria tenalla* and 50,000 of *Eimeria acervulina*. The latter species is but slightly pathogenic in pure infections, but it was used

in connection with the caecal form simply because the writer wanted to see if a more disastrous effect would result from a mixed infection of that type than from pure infections of *E. tenella*. The result was negative so far as could be determined from uncontrolled observations.

All resulting deaths occurred on the fifth and sixth days after inoculation. They were distributed as follows: in the lot on ration 1, 6 out of 17

TABLE 2. *Construction of rations used in experiment 5*

Materials	Ration 1	Ration 2	Ration 3
Yellow corn meal	35	35	35
Meat scraps	3	3	4
Fish meal (dried ATCO)	4	4	4
Soy bean oil meal (expeller process)	13	4	....
Ground hulled oats	21	20	4
Wheat bran	6	6	5
Wheat middlings (gray shorts)	10	10	30
Alfalfa meal (13 per cent protein)	2	2	2
Charcoal, fine	1	1	1
Oyster shell, fine	2	2	2
Salt	1	1	1
Buttermilk, dried	....	10	10
Casein, commercial	0.5	0.5	0.5
Cod liver oil (Nyal)	2	2	2
Total parts by weight	100.5	100.5	100.5
Percentage protein (calculated)	18.4	17.9	17.7

inoculated chicks; in lot on ration 2, 9 out of 17 inoculated chicks; in lot on ration 3, 12 out of 15 inoculated chicks. Chi-square tests showed a significant difference in fatalities occurring in the lots on ration 1 and ration 3. The tests for the lots on rations 1 and 2 and for lots on rations 2 and 3 were not significantly different statistically, possibly because the number of fatalities in each lot was about what one could expect if both dried milk and wheat middlings were culpable.

There was another angle to the effects of the diet, however, that was not measurable in mortality. On the seventh and eighth days of the infection every one of the 8 survivors in the lot on ration 2 stood hunched and motionless with the eyes closed for a considerable part of the time. It seemed almost certain that they would perish. The survivors in the lot on ration 1, on the contrary, were moving about and eating a small amount of food during that same period, save for 2 or 3 which were in a condition resembling all the survivors in the lot on ration 2. All the apparently hopeless cases in the 2 lots, however, made remarkable recoveries. What is the explanation of what occurred? It is probably the acute attack which holds the key to the situation. The more susceptible individuals in the first lot passed away when the hemorrhage was at its height on the fifth and sixth day, but the survivors which received no dried milk and the reduced amount of middlings rapidly passed the crisis. The crisis lasted longer in the lot which received the buttermilk and the reduced amount of middlings, but the infection was not quite heavy enough to kill any more of the survivors. The behavior of the surviving chicks in the lots on rations

1 and 2 gave every indication that a somewhat heavier infective dose would have produced far more casualties in the lot on ration 2 than in the lot on ration 1.

The growth records of the birds are also of interest. During the first 17 days on the special rations, which included the third day of the infection, the lots on rations 1, 2, and 3 made mean weight gains of 110 gm., 131 gm., and 124 gm., respectively. Seventeen days after these weighings were made, the respective further mean weight gains were 117 gm., 161 gm., and 118 gm. The latter figures are based, of course, only on survivors—11 in the first lot, 8 in the second, and 3 in the third. There were 11 hens in the survivors of the first 2 lots which were given to a janitor on condition that he would report on the progress they made. Two months after the last weighing all were still alive and apparently thrifty.

All other survivors were autopsied after the last weighing on the twentieth day of the infection. Some of them still retained caecal plugs as evidence of past infection, but some of the caeca had returned to a condition close to the normal. A study of the fate of these plugs would be a profitable undertaking, but so far as the writers could tell, from casual observation, they had little adverse influence on recovery or weight gains made by the birds.

TABLE 3. *Construction of rations used in experiment 6*

Materials	Ration 1	Ration 2	Ration 3
Yellow corn meal	30	30	30
Wheat middlings	5	25	5
Wheat bran	5	5	5
Ground barley	5	5	5
Oat flour	10	10	10
Ground whole oats	20	....	18
Soy bean oil meal (expeller process)	15	15	15
Oyster shell, fine	2	2	2
Salt	1	1	1
Cod liver oil	1	1	1
Fish meal	6	6	....
Dried buttermilk	....	....	7
Steamed bone meal	....	....	1
Total parts by weight	100	100	100
Percentage protein (calculated)	18.1	18.9	17.1

EXPERIMENT 6

This experiment was planned for testing further the effect of dried buttermilk in the ration and acquiring evidence concerning the possibility that middlings in a ration without dried milk has an unfavorable effect upon caecal coccidiosis. A still more compelling motive was to ascertain whether pullorum infection was being carried along in the cultures of *Eimeria tenella*. Furthermore, although all the chicks previously used were from supposedly pullorum-free stock and none of the dead birds showed the typical lesions of pullorum disease at autopsy, it appeared desirable to make a careful study of the possibility that at least some of

the deaths ordinarily attributed to coccidiosis might be due to pullorum, or at least complicated with it. Since cultures of the tissues of the dead birds would check both possibilities, arrangements were made with Dr. E. F. Waller of the Veterinary Pathology Department at Iowa State College to make autopsies of the dead birds and to attempt to culture the bacterium from the livers. He found that the lesions in the birds that succumbed were not those of pullorum disease, that the condition of the caeca in each case was indicative of death from caecal coccidiosis, and that 2 cultures from the liver of each bird were negative for the typical colonies of the bacterium sought. The writers are convinced that in this experiment pullorum disease was not a complicating factor in death of the chicks.

The chicks were kept on a ration similar to the commercial product previously described for the first week of their lives. On the eighth day they were divided into 3 lots, confined in batteries, and put on the rations described in table 3. Each chick was inoculated directly into the crop with 130,000 sporulated oocysts of *Eimeria tenella* from a two-month-old culture. It was necessary to add dried buttermilk to the extent of 2 per cent to rations 1 and 2 beginning on the date of inoculation, because definite indications of paralysis were commencing to develop in a few of the chicks.

Deaths occurred on the fifth, sixth and seventh days. They were distributed as follows: in the lot on ration 1, 5 out of 34 chicks inoculated; in the lot on ration 2, 5 out of 33 chicks; in the lot on ration 3, containing 7 per cent dried buttermilk, 12 out of 33 chicks. Chi-square tests comparing the first and third lots and the second and third lots showed that the lots compared probably belonged to different populations. Obviously the results in the first and second lots were not significantly different. It was shown, then, that dried buttermilk favored severity and that middling in a ration with no dried milk did not favor severity.

The chicks were weighed individually the day they were started on the test rations, and on the ninth, fourteenth and eighteenth days thereafter, the last weighing being on the fourth day of infection. The mean net gains for the 18 days on rations 1, 2, and 3 were 138.4 gm., 159.5 gm., and 160 gm., respectively.

DISCUSSION

The experiments and results described in the foregoing pages have succeeded in demonstrating that one ration may spare the White Leghorn chick from fatal effects in experimental coccidiosis to a far greater degree than another. An inspection of the formulas used in experiments 1, 2, 3, and 5 reveals that a combination of dried skim milk at the 5 or 10 per cent level and wheat middlings at the 30 per cent level predisposes the host to fatal termination of the infection more than do reduced amounts of these feeding stuffs. It is evident that the combination of the dried milks and middlings at the higher levels in the ordinary type of poultry ration, such as that used for the control rations, permits a condition to develop in the bird that is highly unfavorable for its recovery. It is impossible to state at present the nature of this condition; that is, whether it is an enhanced parasite population or a lowering of the resistance of the host to the unmodified parasite population.

Experiments 4, 5, and 6 represented attempts to ascertain the inde-

pendent rôles played by dried milks, particularly dried buttermilk, and middlings. The results in experiments 4 and 6 were definite in placing part of the responsibility on dried buttermilk, but not so definite in experiment 5. When, however, the results of the three experiments were set up as a single experiment with three degrees of freedom and subjected to the chi-square test (according to the procedure outlined by Snedecor, 1937, pp. 154-156), a value indicating a high degree of significance was obtained. Thus it seems definitely safe to conclude that in rations of the type tested dried buttermilk adversely influences the host's chance of recovery. In a paper now in press Becker and Wilcke have contributed further to this conclusion.

Experiment 6 indicates that the independent effect of middlings is nil in the absence of dried milk from the ration, while experiment 5 is suggestive of the existence of a deleterious effect from an increase in the amount of middlings from 10 per cent to 30 per cent, with dried buttermilk in the ration in the latter case. Admittedly, further investigation is needed on the effect of middlings in the ration both in the absence and presence of dried buttermilk. The same admission holds for the independent rôle of dried skim milk.

None of the rations described in this paper are recommended to the practical poultryman for the purose of reducing losses from caecal coccidiosis in his flocks. Paralysis, and perhaps still other dietary disorders, might bring about greater losses than the parasite. The test ration in experiment 3, which contains 2 per cent dried skim milk, would be by far the safest to try out under practical conditions, but until even this ration is tried out further it would be unsafe to recommend it or similar formulas. Sanitation will probably continue to be the safest practise for controlling coccidiosis and other parasite infections, although an improvement over the present situation should result when more is known concerning formulas that will adequately nourish the host and at the same time mitigate the severity of diseases produced by parasites.

SUMMARY

The combination of dried skim milk and wheat middlings or dried buttermilk and wheat middlings in the ordinary type of chick ration was responsible for a high death rate in White Leghorn chicks experimentally infected with caecal coccidiosis. Dried buttermilk of itself was likewise culpable when fed in the ordinary type of chick ration.

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THE DETERMINATION OF CHARACTERISTICS INVOLVED IN THE ABILITY TO DRIVE AN AUTOMOBILE WITH EMPHASIS ON THE REACTIONS OF THE DRIVER

LeVerne Johnson and J. E. Evans

From the Department of Psychology, Iowa State College

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In the operation of the automobile on the highway, three definite factors are involved: the road, the automobile, and the human element. All accidents may be attributed to one or more of these three factors.

Highway engineers have developed highways based upon the scientific results of engineering research. Automobile manufacturers have modified body construction, steering mechanism and tires, and have extended visibility for night driving by adding scientifically designed lights. In spite of the increasingly stringent state requirements for driving licenses and for periodic inspections, and of the tremendous emphasis placed by state and civic organizations on safe driving, accidents have continued to increase.

There is a common belief that accidents are distributed among drivers according to the law of chance. However, in recent years science has demonstrated that accidents do not just happen. Each has a definite cause. One driver may consider himself as efficient as the other, and may attribute his accidents to bad luck, but the courts may declare such accidents due to carelessness; however, carelessness is not an explanation.

In the main, the psychological study of accidents has been confined to the statistical analysis of factors influencing accident susceptibility. This statistical approach is the investigation of group tendencies. While this method is valuable in determining the causes of accidents in a wholesale way, it offers little in the way of procedures for applying the facts discovered to the individual case. In the present study the approach is clinical in that it deals with the individual and his adjustments.

In the battery of tests developed for the present study, emphasis has been given to reaction time measurements. This is justified by the obvious dependence of good driving upon the appropriate reactions of the driver.

Most visual stimuli received while driving call for the application of the brakes, the turning of the steering wheel, or the acceleration or deceleration of the car. The driver responds to the sound of a horn or the noise of an approaching truck. Even his kinaesthetic sense is involved as body changes are made to accommodate to the car movements. In all of these reactions the time element is an important factor.

It is conceded that a driver may not reveal his real driving defects under test conditions but it is maintained that, if observed sufficiently, there are certain habitual characteristics which will manifest themselves.

This study is an attempt to select certain tests or measurements which may be used in the laboratory to determine the type of driving ability which a person taking the tests may possess, or of which he is capable.

The experimental outdoor driving course was used to determine,

under controlled conditions, the actual driving ability of the person tested. This course will be referred to in the future as the "experimental driving course." If there is a high degree of relationship between the performance on the experimental driving course and the achievement in the laboratory tests, it would be possible to use the laboratory tests to measure actual or potential driving ability. The use of laboratory tests will simplify the testing program, reduce the time and expense necessary in making tests, make possible better controlled conditions under which to give the tests, and add to the ease of training experimenters.

This investigation was undertaken primarily to determine whether the ability to drive an automobile can be determined by laboratory tests. Each driver was observed carefully while driving over a prescribed test route four miles in length and then taken into the laboratory and subjected to the following tests:

1. Simple reaction time with light as a stimulus.
2. Simple reaction time in the presence of a high frequency sound of 12,333 vibrations.
3. Simple reaction time following an attempt to induce fatigue through use of the pursuitmeter for 10 minutes.
4. Choice reaction time with lights as stimuli.
5. Foot braking time with light as a stimulus.
6. Foot reaction time with light as a stimulus.
7. Speed-of-foot movement.
8. Accommodation time to light and to darkness.
9. Measurement of ocular dominance.

Three experimental units are involved in this research. One represents the investigation of 14 subjects who made a total of 5,200 reactions, 100 at each sitting. Two of these subjects made 1,000 reactions each, while the other made from 300 to 600 reactions. This information was used for standardization purposes.

The second unit constitutes the information secured on 140 United States Engineers in the War Department and located in the arsenal at Rock Island, Illinois. Of the 140 drivers, 18 were chauffeurs. Most of these 140 engineers, due to selection, training and daily work in driving cars, taxis and trucks, should be good drivers without exception, although the tests did not indicate that this was true in all cases. These were tested during January, 1936. While 247 engineers were tested, only the results on the 140 drivers have been used here except in the computation of the reliability of the tests in which the test results of all the 247 subjects were used. The age range of the engineers was from 19 years to 61 years.

The third unit constitutes the information secured on 38 subjects at Iowa State College between September, 1935 and May, 1936. Of these, 27 were undergraduate college students, 3 were graduate students, 4 were high school students, and 4 were housewives. The age range was from 18 years to 52 years.

With the exception of choice reaction time, the same tests were given to the subjects in the second and third units. Because of physical conditions, the two experimental driving courses were not identical but they were comparable in every way. While the tests given to the engineers

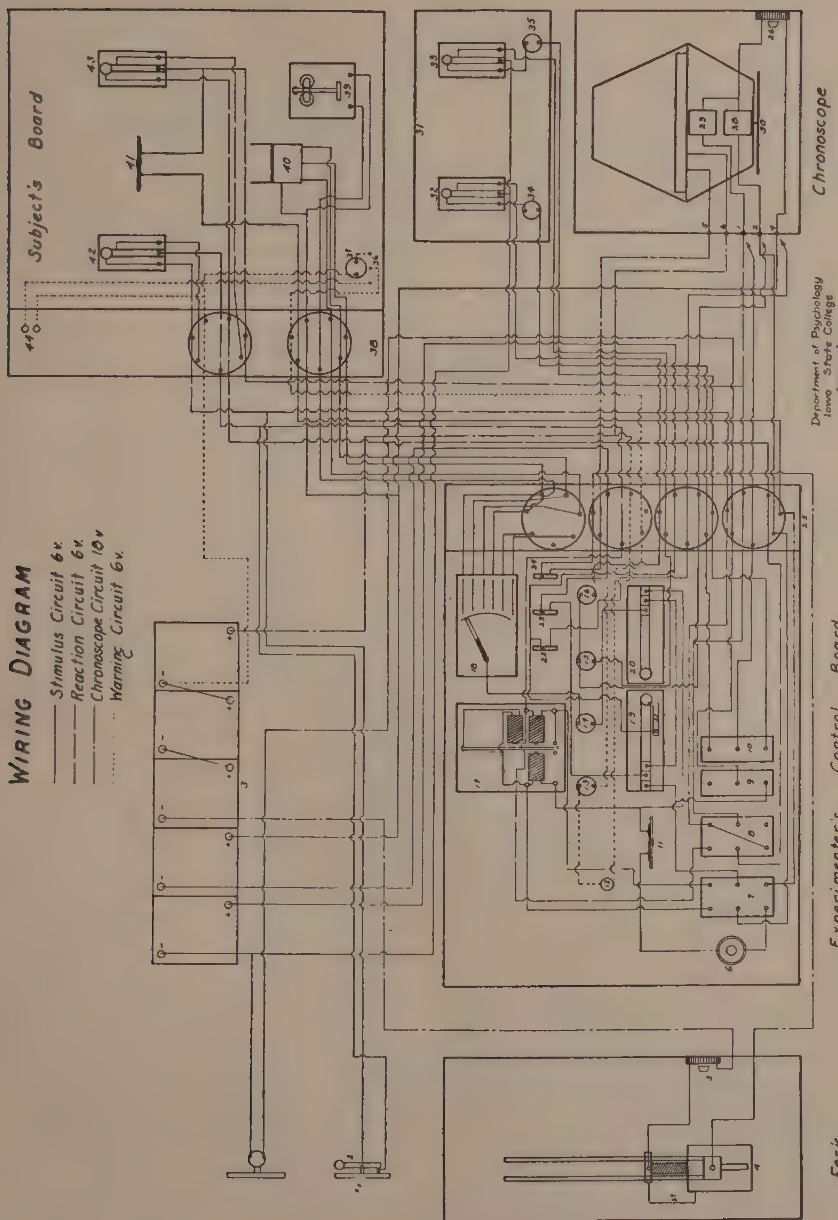

Wiring Diagram
Stimulus Circuit 6v.
Reaction Circuit 6v.
Chronoscope Circuit 10v
Warning Circuit 6v.
Subject's Board
Chronoscope
Experimenter's Control Board
Fork
Department of Psychology
Iowa State College
Ames, Iowa

Fig. 1

were well controlled, conditions in the laboratory at Iowa State College were somewhat better controlled.

APPARATUS AND PROCEDURE USED

For both the simple and the choice reactions the Dunlap chronoscope[1] was used with the Iowa State College group, while the Ewald, set up in the conventional form, was used with the United States Engineers group.

1. *Simple reaction time.*[2] After five practice trials each subject in the United States Engineers group was given a series of 30 reactions. The subject sat at a table and reacted to a red light eight feet directly in front of him, by releasing the reaction key. A "ready" signal was given verbally from one to three seconds before the stimulus, timed by watching a stop watch. The Iowa State College subjects reacted to a white light placed 20 inches away in front of him. (Fig. 1)

2. *Simple reaction time with a high frequency sound of 12,333 vibrations.* This test was the same as the simple reaction time test except that a high frequency sound was introduced. The sound apparatus was lo-

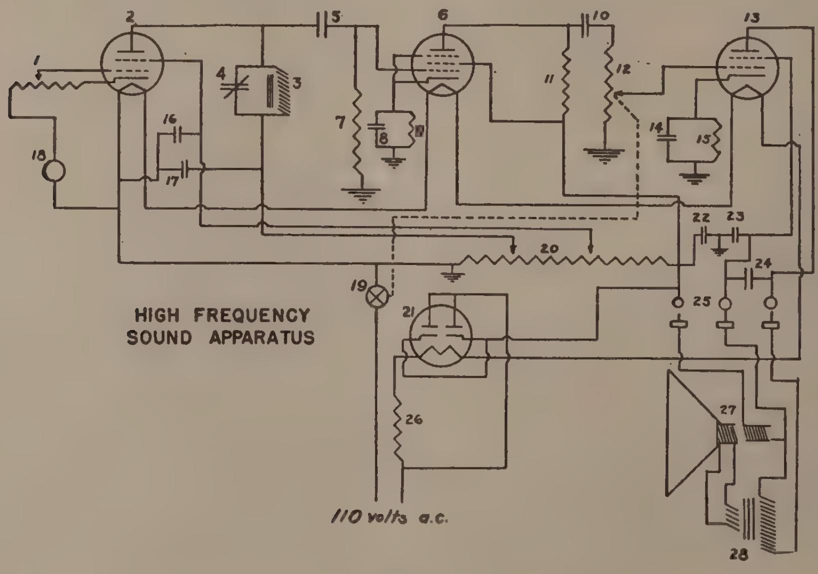


Fig. 2

cated eight feet directly behind the subject. With the subject present the sound was produced for 30 seconds before the reaction series began. A series of 100 reactions was taken with a rest period of 30 seconds after the first 50 reactions. The high frequency sound continued during the rest period. The wiring diagram for the high-frequency sound apparatus is

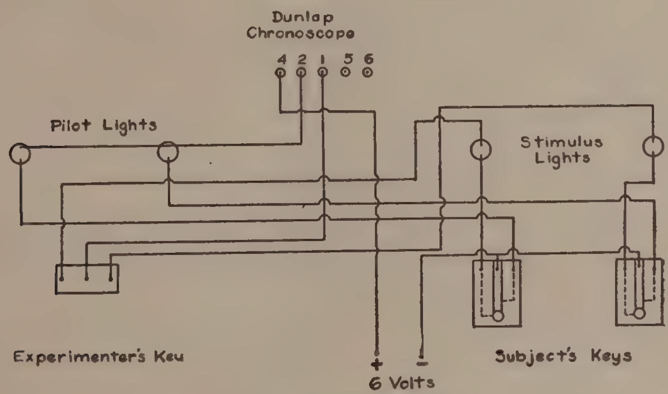
[1] This apparatus was made possible by a gift from the National Research Council.

[2] In all cases, 100 reactions were obtained from the Iowa State College group, and 30 from the U. S. Engineers group.

indicated in figure 2. This test was not given to the United States Engineers group.

3. *Simple reaction time following the pursuitmeter test.* The Meyer Pursuitmeter modified by Weiss and Renshaw was used in an unsuccessful attempt to induce enough fatigue to modify the simple reaction time of the subject.

In the first series of movements the subject followed the pursuit movements by attempting to keep the electric terminal of the stylus in contact with the terminal of the moving bar. When contact was lost, the bar would come to a complete stop in 2.5 seconds. The score was negative and represented the number of times the contact was lost. This series required 5 minutes.



CHOICE REACTION TIME

Fig. 3

The last series of movements consisted of holding the electrode of the stylus inside a one-half inch hole which moved with the moving bar. When the stylus touched the side of the hole an error was registered. If the stylus rode the side of the hole, the bar stopped. The last series of movements lasting 5 minutes followed the first with a five-second interval between for changing the switches. With the exception of the five-second interval, the subject was required to concentrate intently upon the pursuit movements during the entire 10 minutes.

4. *Choice reaction time.* The subject reacted by removing his right index finger from the right key, or his left index finger from the left reaction key, depending upon whether the light stimulus appeared at the right or at the left of the subject. The stimulus was 20 inches away from the subject. This test was not given to the United States Engineers. A series of 100 reactions was obtained from each subject with a rest period of 30 second at the end of 50 reactions. A series of 25 presentations, 13 left and 12 right, in random order, was given and then repeated. (Fig. 3)

5. *Foot braking time.* Each subject was given the following instructions: "This test is to determine the time needed by you to move your foot from the accelerator pedal to the brake pedal and to depress this brake to the floor board. Push the accelerator down until a buzz is heard.

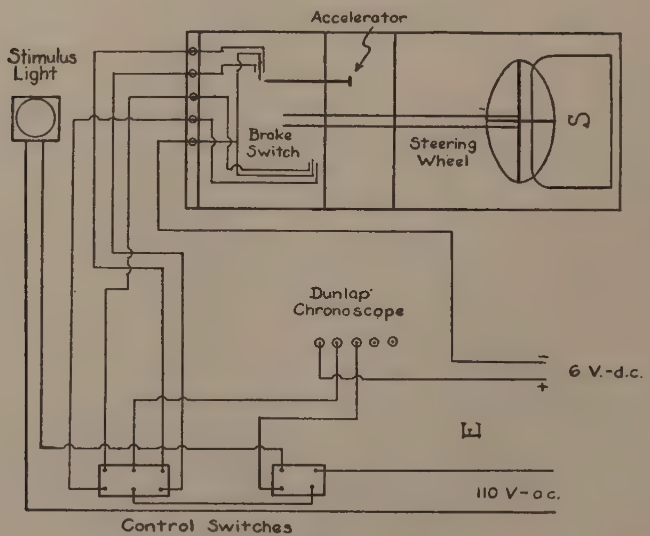
Hold it down until the red stimulus light appears. When the red light appears, put the brake on as quickly as you can by pushing the brake pedal down until a dull buzz is heard under the seat where you are sitting. In about five seconds after each reaction, place your foot back on the accelerator to be ready for the next reaction." Pressure used on the pedal was determined by taking the average brake pedal pressure on 12 standard makes of cars. This amounted to 32 pounds.

After giving detailed instructions and two practice trials, 25 reactions were made by each subject. The stimulus, a red light, was set 10 feet directly in front of the subject. No "ready" or warning signal was given, except at the beginning of the series.

6. *Foot reaction time.* After the test of foot braking time, the following additional instructions were given for the simple foot reaction time: "The principle is the same in this test except that you simply remove your foot from the accelerator pedal as quickly as you can after the red light appears. Concentrate upon the red light."

Two practice trials were given. A series of 25 reactions was taken. The stimulus light was in the same position as for the foot-braking-time test and no "ready" signal was given.

The accessory part of the foot-braking-time and foot reaction time apparatus consisted of a steel frame fitted with a steering wheel, clutch, brake and accelerator pedals, and an adjustable seat. (Fig. 4) Both the brake and accelerator pedals were attached to switches for starting and stopping the chronoscope. The steering wheel was rigid and was used only for the subject to grasp. When the accelerator was depressed a small buzzer sounded, indicating to both the subject and the operator that the circuit was completed. Likewise, the brake pedal was equipped with



FOOT-BRAKING TIME APPARATUS

Fig. 4

a buzzer to indicate when it was depressed sufficiently to complete the reaction circuit and to make the test uniform for all subjects.

The visual stimulus was a 20 watt, 110-volt bulb mounted behind a red tail light lens four inches in diameter and set 10 feet in front of the subject.

7. *Speed-of-foot movement.* A foot tapping apparatus was used. Two small pedals, similar to the accelerator pedal of an automobile, were placed on a board inclined at an angle of 30 degrees with the floor. The two pedals were placed seven inches apart and held in position by a small coil spring around the shaft of each. The right pedal was attached to a Veeder counter. Timing was done with a stop watch.

The following instructions were given: "This is a test to determine the speed at which you can move your foot between the two pedals. With the right foot push first one pedal and then the other as fast as you can. The test will continue for two minutes. Continue tapping until told to stop."

8. *Accommodation to light and to darkness.* In this apparatus the light was produced by a standard 1936 Ford headlight with a 32-candle power bulb. The light was focused directly into the subject's eyes from a distance of 20 feet. At this distance the light was just slightly more than 7 foot candle power. The light was operated from a separate battery by means of two relays. One relay turned the light on when the subject was accommodating to light, and the other turned the light off when the subject was accommodating to darkness. Both parts of the accommodation tests were performed in a semi-darkened room.

The stimulus lights projected the letters "R" or "L" on a ground-glass on the front of the box containing the stimulus lights. The subject reacted to the letters by releasing the corresponding right or left hand key, depending upon which letter was revealed.

The height of the stimulus letters was 1.25 inches. According to the Snellen visual acuity chart, such letters are visible to persons with vision as low as 29 per cent at a distance of 20 feet. Each letter always appeared at the same place on the ground glass, which prevented the subject from getting any cue from the position of the letter.

The subject was seated 20 feet from the headlight and directly in front of the stimulus box. The stimulus box was located the same distance away and two feet to the right of the headlight. The following instructions were given the subject: "This test is to determine how long it will take you to accommodate to a bright light shining directly into your eyes. Look at the stimulus box all of the time. Do not change the position of your head. Keep both keys before you depressed. The headlight and the stimulus light will appear at the same time. As soon as you can read the letter "R" or "L" on the glass in the front of the stimulus box, react by removing the right or left hand from the key, depending upon whether the "R" or "L" has appeared."

The test for accommodation to darkness followed after a rest period of two minutes in a semi-darkened room. The following instructions were given: "This time the light will shine between reactions and you react to the same stimulus as before, when the light from the headlight disappears." As the stimulus lights and the relay operating the headlight were wired in series, the headlight disappeared and the stimulus letter appeared simultaneously.

A series of 10 measurements was taken for each type of accommodation, five to "R" and five to "L". A chance order was followed. (Fig. 5)

9. *Determination of ocular dominance.* A hollow truncated cone 14 inches long and slightly flattened and with a hole at the small end, 0.125 inches in diameter, was used. The cone was mounted on a rigid base. The subject looked through the large end of the cone at a white vertical line ¼ of an inch wide on a black background five feet from the end of the cone.

By the use of such a cone, unilateral vision was forced, although the subject usually thought he was seeing with both eyes. The small end of this ocular dominance cone was directed by the experimenter at a point 18 inches to one side or the other of the white line, then the subject was instructed to swing the cone back until he could see the white vertical line. This was repeated for the left side. Six trials were given on each side.

10. *The experimental driving course.* Although a driving history of each subject was obtained, it was felt that this history was not sufficiently adequate, as many subjects seemed reluctant to reveal accidents which we knew they had previously had, or to admit that the accident was due to their inability to drive well. However, what information was obtained correlated closely with the results on the driving field, and with the results of the laboratory tests.

To determine the driving ability of the subjects under uniform conditions, an experimental driving course was used. This test was very similar for both the United States Engineers and the Iowa State College group, although given on different driving courses.

The achievement of the subjects on the experimental driving course constitutes the criterion of driving ability. Those classified in the lower

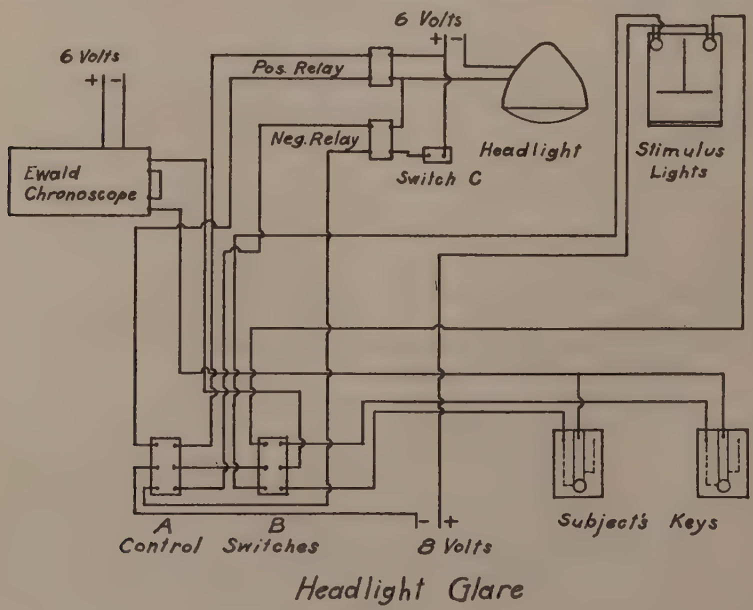


Fig. 5

fifty percent of the group were designated as 'poor', while those in the upper fifty per cent were termed 'good' drivers. All the observations and scoring of drivers on the outdoor driving course was done by one person and all conditions kept uniform. The driving rating scale used is found in appendix I.

The test results obtained in the laboratory were checked against this driving field criterion. While such a criterion cannot be absolutely accurate, it is very difficult, if not impossible, to secure a more reliable criterion.

In laying out the experimental driving course for this study, the attempt was made to include as many of the conditions of everyday driving as possible. The four-mile course included many of the natural hazards found in driving. To these natural hazards several artificial ones were added. (Fig. 6)

Three miles of the course were concrete paved and the remaining mile was a single lane road surfaced with cinders. There were six left turns, seven right turns and three busy intersections. One block was of heavy traffic and the subject entered it with a right turn. He was required to cross the traffic by turning left. Three stop signs used by regular traffic were included. One of these was at a busy street intersection, one was encountered upon entering a heavily travelled street, (Lincoln Highway) and the other was at a less frequently travelled intersection. One corner was obstructed by a high bluff around which the subject could not see until at the intersection line. A hidden horn was located behind the bluff and was sounded as the subject reached a point 100 feet from the corner.

Before starting the outdoor test each subject was told briefly that the results of his outdoor driving test would be compared with the results of the tests taken in the laboratory. After this brief explanation, each subject was given the following instructions: "You are to drive as you ordinarily do. You will drive over a selected driving course which I shall indicate to you as we drive along." The subject then began the trip over the designated course. The observer rode in the front seat with the subject. No mention was made of the natural or artificial hazards to be encountered. All possible observations were made of the subject and recorded on the data sheet by the observer. No information which might influence the driving was given in response to the questions of the subject. The subject secured no information from the data sheet.[3]

During the Iowa State College experiment, an additional car was used to increase the number of hazards. This car was in charge of the same assistant at all times. The assistant's car always started a few minutes ahead of the test car, but the subject did not know in the beginning that this car would participate in the experiment.

RELIABILITY OF TESTS USED

1. *Simple reaction time.* A series of 30 reactions was taken and the reliability calculated by correlating the means of the first 15 measures with the means of the last 15 measures. The coefficient obtained was .85. Highly significant point is .208. (6) According to Fisher, a correlation coefficient of .304 is significant and one of .393 is highly significant for a

[3] A set of directions for using the driving rating scale, with a detailed statement of the driving procedure will be supplied upon request.

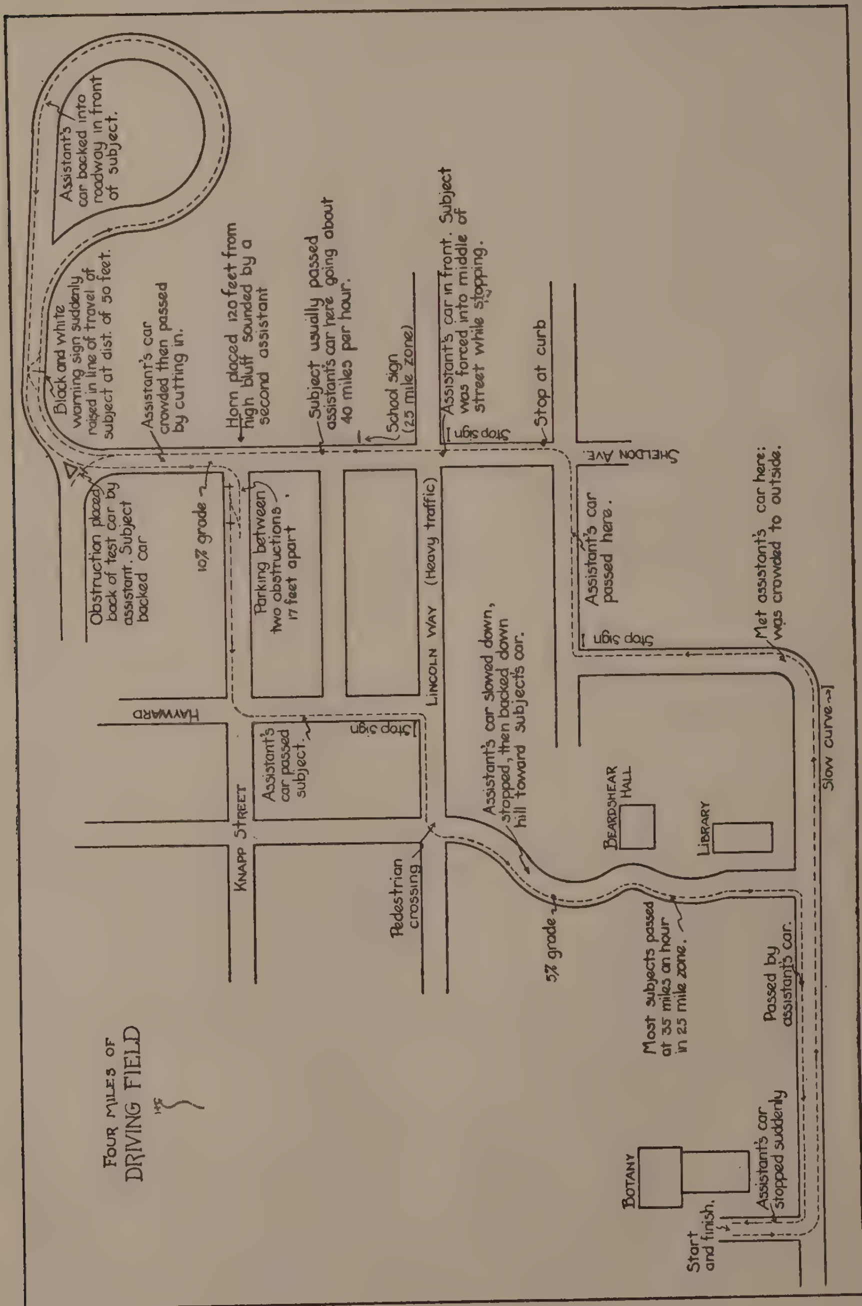

Assistant's car backed into roadway in front of subject.
Black and white warning sign suddenly raised in line of travel of subject at dist. of 50 feet.
Assistant's car crowded then passed by cutting in.
Horn placed 120 feet from high bluff sounded by a second assistant
Subject usually passed assistant's car here going about 40 miles per hour.
School sign (25 mile zone)
Assistant's car in front. Subject was forced into middle of street while stopping.
Stop sign
Stop at curb
Sheldon Ave.
Obstruction placed back of test car by assistant. Subject backed car
10% grade
Parking between two obstructions 17 feet apart.
Lincoln Way (Heavy traffic)
Assistant's car passed here.
Stop sign
Met assistant's car here; was crowded to outside.
Hayward
Assistant's car passed subject.
Stop sign
Assistant's car slowed down, stopped, then backed down hill toward subject's car.
Beardshear Hall
Library
Slow curve
Knapp Street
Pedestrian crossing
5% grade
Most subjects passed at 35 miles an hour in 25 mile zone.
Passed by assistant's car.
Four miles of Driving Field
Botany
Assistant's car stopped suddenly
Start and finish.

Fig. 6

group of 40 samples. This would indicate that the above tests are of sufficient reliability at least for group comparisons.

2, 3, 4. *Simple reaction time, choice reaction time, simple reaction time after the pursuitmeter test, and simple reaction time with high frequency sound.* For the Iowa State College group the reliability coefficient of these tests was obtained by correlating the means of the first 50 reactions with the second 50 reactions.

5. *Foot braking time test.* In measuring the speed of movement from accelerator to brake, 25 measures were taken for each subject. The reliability was computed by correlating the means of the first 13 measures with the means of the last 12 measures. The reliability was .82.

6. *Foot reaction time.* A series of 25 reactions was taken and the reliability calculated by correlating the means of the first 13 measures with the means of the last 12 measures. The coefficient obtained was .78.

7. *Speed-of-foot movement test.* This test was given twice to 22 subjects. The second trial was given not more than two days later than the first and within and hour of the same time of day. The reliability coefficient was .77. The effect of practice probably had an influence although each subject had an equal chance of improvement on the second trial.

8. *Accommodation time to light and to darkness.* Due to the nature of the test and the limited number of measurements made because of excessive eye strain, the method of correlation did not seem adequate for determining the reliability of the accommodation measurements. For that reason an analysis of variance was calculated. It is obvious that if the scores on a test vary more within the range of the individual's scores than between the means of the individuals tested, it is of little statistical value. The two following tables show the results of the analysis. A single criterion of classification with equal numbers of observations in the classes was used.

TABLE 1. *Analysis of variance*
Accommodation to light

Source	D.F.	Sum of Squares	Mean Square
Total	119	144,414	1,213.35
Between means	11	102,446	9,313.27
Within groups	108	41,968	388.59

$F = 9{,}313.27/388.59 = 23.96$ F must equal 2.69 to be significant.

Statistically a value of F as high as the above value could not be attributed to random sampling. An actual difference exists. There is less variation within each individual's scores on the average than between the means of different individuals.

TABLE 2. *Analysis of variance*
Accomodation to darkness

Source	D.F.	Sum of Squares	Mean Square
Total	119	37,126	311.98
Between means	11	22,416	2,037.81
Within means	108	14,710	136.20

$F = 2{,}037.71/136.20 = 14.96$ F must equal 2.69 to be significant.

As stated for the value of F in the previous table, it is evident that an F-value as high as the above cannot be attributed to random sampling. The score of any subject does not vary significantly within itself but the individual mean scores vary from one subject to the other.

9. *Measurement of ocular dominance.* Since the test results of ocular dominance were not expressed in numerical figures, they were not included in the previous statistical analysis. Of the group there were six subjects who were dominant in one eye. Four were dominant in the right eye and two in the left. The mean score on the driving test for this group was 15.82 which is insignificantly higher than the mean for the entire group.

10. *Experimental driving course test.* Due to the type of test and the number of subjects used, no attempt was made to give the test more than once to each driver. In order to obtain a measure of reliability, the data sheet was divided into two parts, and the mean of the first half correlated with the mean of the second half. The reliability coefficient obtained was .56 with the 247 United States Engineers, and .682 with the Iowa State College group of 38 cases. Highly significant point is .160. (6)

11. *The preliminary experiment.* The preliminary experiment was performed on 14 subjects for the primary purpose of determining the number of reaction measurements necessary to insure an accurate index to the subject's speed of reacting, and for improving the reaction time technique to be used in the later units of this research. The only measurement taken was simple reaction time to a visual stimulus. The same apparatus under the same conditions was used in this experiment as in the Iowa State College unit.

Each subject was given 10 preliminary reactions before the series began. At each sitting 100 reactions were taken with a rest period of 30 seconds after the first 50 reactions.

All tests were given in the afternoon usually between the hours of four and six o'clock, and no subject was given more than 100 reactions at one sitting. A total number of 5,200 reactions was taken. Two subjects gave 1,000 reactions each and the others from 300 to 600 reactions.

To obtain the reliability of the test the first 50 measurements were correlated with the second 50 for each trial. The Pearson products-moment method was used, with the modified Ayers formula. A correlation coefficient of .92 was obtained, which is highly significant. Significant values of r for 50 trials = .273 to .354. (6)

The mean of the first 50 reactions for each subject was .216 seconds. The mean of the second 50 reactions for each subject was .219 seconds. The following table shows the results of the analysis of the difference.

TABLE 3. *Analysis of reaction times*

Series	Number Trials	Means	S. D.
First 50	52	.216 sec.	28.71
Second 50	52	.219 sec.	29.10

Mean difference = .003. S.D. difference = 5.72.
S. D. = 4.01 for first 50, and 4.08 for second 50.

$$T = \frac{.003}{5.72} = .00052 \text{ (insignificant).}$$

T should be at least 2.008 to be significant. (6)

From these data it is evident that there is little difference between the first 50 reactions and the second 50 reactions. This is more evident when one considers the fact that the reliability of the test was .92. However, the small difference was quite consistent. In 81 per cent of the cases the average time for the second 50 reactions was longer than for the first 50 reactions. This might indicate possible fatigue, or ennui.

In order to determine the number of reactions necessary to obtain an accurate measure a curve was constructed. Points were plotted for from 50 to 1,000 reactions. The data indicate that the subject reaches his 'reacting stride' after making about 300 reactions.

TABLE 4. *Summary of reliability of tests used*

	Iowa State College Group		U. S. Engineers Group	
Test	Reliability Coefficent	Number Cases	Reliability Coefficient	Number Cases
Simple reaction time	.901	38	....	....
Simple reaction time with high frequency sound	.843	38	....	....
Simple reaction time after pursuitmeter	.876	38	....	....
Choice reaction time	.844	38	....	....
Braking time	.736	38	....	....
Foot reaction time	.801	38	.82	247
Experimental driving course	.682	43	.56	247
Speed-of-foot movement	....	......	.77	22

Accommodation time to light and darkness . See pages 18 and 19.
Measurement of ocular dominance .... See 9, on page 20.

STATISTICAL RESULTS

1. For comparing the experimental driving scores with the scores on the other tests, a multiple correlation coefficient was calculated. The method used was that described by Wallace and Snedecor. (12) Table 5 shows the zero order correlation coefficients found in the process of calculating the multiple correlation.

TABLE 5. *Zero order correlation coefficients*

	A	B	C	D	E	X
A		—.1046	—.1326	—.0867	.2188*	.2163*
B			.4637*	.2840*	—.2393*	.2319*
C				.5529*	—.4693*	.1435
D					—.3901*	.1724*
E						—.1667*
X						

* Significant values of r.

A = Experimental driving.
B = Foot braking time.
C = Foot reaction time.
D = Simple reaction time.
E = Speed-of-foot movement.
X = Age.

While the multiple correlation is barely significant for a comparison involving six variables it is of little predictive value.

The experimental driving test scores gave significant correlations with only two variables; speed-of-foot movement, and age. The effect of age was eliminated by partial correlation from all the comparisons involving experimental driving with the following results. All partial correlations were calculated by the formula:

$$r_{12.3} = \frac{r_{12} - r_{13}r_{23}}{(1 - r_{13}^2)\ (1 - r_{23}^2)}$$

TABLE 6. *Correlations*

Zero Order Correlation	Partial Correlation
$r_{AB} = -.1046$	$r_{AB.X} = .0546$
$r_{AC} = -.1326$	$r_{AC.X} = .1040$
$r_{AD} = -.0867$	$r_{AD.X} = .0806$
$r_{AE} = +.2188$	$r_{AE.X} = .1904$

The fact that $r_{AB.X}$ is much lower than r_{AB} is of little importance, due to the fact that neither relationship is significant. The same is true of r_{AC} and r_{AD}. The only significant value, $r = .2188$, was not lowered significantly.

TABLE 7. *Test score of eighteen chauffeurs*

A	B	C	D	E	F	M
1	18.10	12.6	15.8	32.0	187	29
2	20.60	11.8	13.5	28.2	200	27
3	19.79	12.8	16.4	36.7	173	24
4	18.06	13.4	12.4	27.4	211	28
5	19.11	12.4	11.7	27.8	202	34
6	18.08	14.2	17.3	28.2	201	29
7	17.96	13.4	15.1	31.0	189	33
8	20.60	14.4	19.5	41.8	193	29
9	19.00	13.3	14.8	29.9	211	30
10	17.77	12.9	13.8	31.0	188	30
11	19.05	13.9	14.4	31.0	194	34
12	19.07	11.9	15.1	37.8	213	24
13	19.61	14.4	17.2	38.3	195	29
14	17.32	12.5	15.2	35.6	180	22
15	19.14	11.7	12.7	42.9	198	38
16	19.17	12.0	12.8	28.4	221	31
17	19.37	13.3	11.9	30.4	252	26
18	18.57	14.3	15.0	35.1	165	27
Means	18.91	13.0	14.7	32.9	204.2	29.1
Sum of squares (corrected)	32.28	26.20	84.02	449.30	6,442.50	
Means (remainder of group)	17.50	13.2	14.9	34.2	199.2	33.0
Sum of squares (corrected)	394.49	485.20	658.28	3,063.77	83,302.62	
Pooled sum of squares	426.77	511.40	742.30	3,512.07	89,725.12	
Variance	2.74	3.28	4.76	22.48	575.16	
Variance, mean difference	1.72	2.06	3.98	14.09	360.57	
Standard deviation, mean difference	1.31	1.43	1.99	3.75	18.98	
$t =$	1.08	.140	.101	.035	.282	
$p =$	.30	.85	.90	.90	.75	

2. In the group of 140 drivers at the arsenal there were 18 chauffeurs. These were separated from the remainder of the group and their scores are shown in table 8.

From the above data it is evident that there was little statistical difference shown between the scores made by the chauffeurs and the scores made by the remainder of the group.

The greatest difference was shown between the means of the experimental driving test scores. When t equals 1.08, p equals .30 for infinite values of n. Three times out of ten t would be expected to exceed 1.08 in a random sample. But this is not sufficient evidence to assert that the chauffeurs made better scores than the remainder of the group. This is to be expected, as the entire group of 140 was carefully selected and anyone who showed an accident record was transferred to work in which driving was not required.

When the means of braking time, foot reaction time, simple reaction time and speed-of-foot movement were compared, p was .75 or greater in each case. Such a statistical control is little better than chance and of no predictive value.

However, scores made by the chauffeurs were consistently better than those made by the remainder of the group. This shows some indication of the superiority of the former group.

3. Table 8 shows the zero order correlation coefficients for the entire Iowa State College unit. Driving ability gave significant correlations with only three tests, namely, choice reaction time, foot reaction time, and speed-of-foot movement. This relationship was changed somewhat after the solution of the normal equations to obtain the betas.

TABLE 8. *Correlation coefficients—Iowa State College unit*

	B	C	D	E	F	G	H	I	J
A	—.234	—.442*	—.212	—.246	—.148	—.113	—.144	—.313*	.307*
B		—.712	.840*	.812	.141	.277	.637*	.855*	—.482*
C			.617*	.632*	.312*	.341*	.800*	.832*	—.522*
D				.844*	.201	.197	.621*	.824*	—.466*
E					.156	.204	.577*	.796*	—.555*
F						.772*	.211	.114	—.043
G							.276	.208	—.009
H								.744*	—.552*
I									—.477*

* Significant values of r. r should equal .304 to be significant and .393 to be highly significant.

A. Experimental driving course.
B. Simple reaction time.
C. Choice reaction time.
D. Reaction time following pursuitmeter.
E. Reaction time with sound distraction.
F. Accommodation to light.
G. Accommodation to darkness.
H. Foot braking time.
I. Foot reaction time.
J. Speed-of-foot movement.

$\text{Beta}_{AB} = .200$ $\text{Beta}_{AD} = .197$ $\text{Beta}_{AH} = .668$ $\text{Beta}_{AJ} = .227$
$\text{Beta}_{AC} = .983$ $\text{Beta}_{AE} = .052$ $\text{Beta}_{AI} = .085$

These values gave a multiple correlation of .603 which is significant. (6) It is not highly valuable for individual comparisons but is adequate for group comparisons. From the above figures it may be noted that the reaction time following the pursuitmeter test, reaction time with sound distraction, and foot reaction time do not contribute any strength to the regression equation. By recalculating the normal equations and using the remaining variables a multiple correlation coefficient of .601 was obtained. This small reduction in R indicates that no significant value was lost by eliminating the three variables mentioned. After examining the zero-order correlation coefficients and betas, it seems that the one test of the entire group that best predicts driving ability is choice reaction time.

4. The mean of the simple reaction time after the pursuitmeter test was .2363 seconds while the mean for the simple reaction time was .2335 seconds. The small difference of .0028 is very insignificant, the *t-value* being only .012. This would indicate that the pursuitmeter test had very little or no influence on the second series of simple reaction time measurements. In selecting the pursuitmeter test it was assumed that the test might induce a state of fatigue, since a high degree of concentration and motor control is necessary to perform the test. The final results did not justify the assumption.

5. The mean of the reaction time with the high frequency sound was .2453. The difference between this and the simple reaction time was .0118. The standard deviation of the difference was .0052 which gives a *t-value* of 2.233. This value is significant but not highly significant. Points of significance are from 2.021 to 2.704. (6) This indicates that on the average the subject's simple reactions were slower when accompanied by the high frequency sound.

6. Premature reactions. Record was made of the premature reactions for the simple reaction time, the reaction time following the pursuitmeter, and the reaction time with the high frequency sound present. The average number of premature reactions per 100 reactions was as follows:

Simple reaction time	2.6
Reaction time after pursuitmeter	2.7
Reaction time with high frequency sound	4.2

There is no significant difference between the number of premature reactions made during the simple reaction time and the number made during the reaction time after the pursuitmeter. The *t-value* was .004.

The difference between the premature reactions made during the simple reaction time and during the reaction time with the sound present is significant, the *t-value* being 5.26.

The number of errors, response made with the wrong hand or with both hands, made during the choice reaction time test was also recorded. The mean per 100 reactions was 1.6.

A further analysis of the premature reactions and the errors made on the choice reaction time was used in comparing the "good" and the "poor" drivers. The following table gives the comparison.

In simple reaction time the "poor" drivers made 3.6 times as many premature reactions as the "good" drivers. In reaction time following the pursuitmeter test the ratio was 1.0 to 1.5, and in reaction time with a high frequency sound it was 1.0 to 1.9. The "poor" drivers also made 1.5 times as many errors on the choice reaction time test.

TABLE 9. *Comparison of drivers*

Premature reactions per 100 during	"Good" Drivers	"Poor" Drivers
Simple reaction time	1.2 S. D. 1.7	4.3 S. D. 1.6
Reaction time after pursuitmeter	2.2 S. D. 2.1	3.3 S. D. 1.8
Reaction time with high frequency sound	3.0 S. D. 1.4	5.6 S. D. 1.6
Errors per 100 during choice reactions	1.3 S. D. 0.8	2.0 S. D. 0.9

7. The tests of visual accommodation, while they gave no significant correlations with any other test, yielded results on speed of accommodation to direct light and to darkness. The average time taken to accommodate to direct light was 1.46 seconds. The average time for accommodating to darkness was .734 seconds, or about half as long as the accommodation time to light. The variability for accommodation to light is three times as great as the variability for accommodation to darkness.

It is significant to note that the time taken to accommodate to darkness, which is what often happens after passing a car at night on the highway, is .734 seconds, during which time a car travelling 60 miles per hour would travel 64.6 feet. If the driver is forced to drive at the same speed with the bright light from an approaching car in his eyes, he would travel at least 129 feet before his eyes could accommodate to the light.

SUMMARY

1. The combination of tests given the United States Engineers which gave the best prediction was foot reaction time, speed-of-foot movement, and age. However, the foot reaction time did not have a highly significant value.

2. A group of chauffeurs was isolated from the group tested for the United States Engineers and the differences analyzed. The only test in which the chauffeurs differed significantly from the remainder of the group was in the experimental driving course test. In outdoor driving they made, on the average, 8.2 per cent better scores.

3. In the Iowa State College group the four tests, simple reaction time, choice reaction time, braking time, and speed-of-foot-movement gave the highest correlation with the ratings on the experimental driving course.

4. The highest correlation of driving ability with any other test was with choice reaction time. The multiple R, using the four tests mentioned above was .601. The correlation of driving ability with choice reaction time was —.442.

5. Of greater significance than the actual time on the reaction time tests was the number of premature reactions made during the reaction time tests. It was found that the drivers whose scores were below the mean on the experimental driving course test made, on the average, 2.0 times as many premature reactions as those whose scores were above the mean.

6. The tests of visual accommodation time gave no significant correlation with any other test. This may have been because nothing comparable was included in the driving test. As the driving test was given entirely in the daytime, there was no chance for the experimenter to deter-

mine whether the subjects were susceptible to the conditions which would appear during night driving. Visual accommodation to light is 2.0 times as long as accommodation to darkness. Accommodation to light is almost 3.0 times more variable.

7. The pursuitmeter test as used for this study has but little effect upon the speed of simple reaction time. ($r = .442$).

8. Ocular dominance gave no significant results with the group tested. In the group there were only six persons with one dominant eye. This was too small a sample for any reliable evaluation.

9. An observation of importance does not show in the statistical results. This was the definite inability of all the 38 Iowa State College subjects to localize the sound of the Delco-Remy horns placed around the corner behind the high bluff in the driving course. Some drivers thought the warning was the horn on the assistant's car which was behind at the time. Others thought the sound might be the horn of some other car approaching from behind, although no car was behind except the assistant's car. Some were unable to localize the sound anywhere. In spite of this inability, all except 3 of the drivers arrived at the corner going 40 miles per hour. As the intersecting streets were narrow, a head-on collision would have occurred if another car had come around the corner.

APPENDIX I

RECORDS OF U. S. ENGINEERS

A	B	C	D	E	F	M
1	16.26	16.1	18.0	33.7	204	46
2	15.50	12.3	14.5	29.3	218	26
3	19.14	11.6	14.8	33.5	248	26
4	17.63	13.2	14.2	37.6	205	32
5	17.39	11.7	13.5	25.1	172	28
6	18.85	17.0	17.9	36.8	191	32
7	17.69	13.7	14.4	37.0	164	28
8	15.30	11.9	14.8	34.1	198	21
9	20.60	11.8	13.5	28.2	200	27
10	18.10	12.6	15.8	32.6	187	29
11	18.27	12.3	14.7	32.2	174	31
12	17.12	10.8	13.5	37.7	193	37
13	18.03	11.7	13.7	41.5	193	25
14	17.88	22.2	22.6	42.0	130	42
15	15.00	11.7	13.0	30.5	188	25
16	19.40	15.2	18.6	45.0	197	30
17	16.66	15.9	14.0	31.3	203	22
18	19.82	12.1	15.4	35.6	209	28
19	15.89	12.6	13.3	30.3	165	26
20	18.26	11.8	12.5	25.5	209	26
21	18.53	13.0	15.5	29.5	216	26
22	19.79	12.8	16.4	36.7	173	24
23	16.92	12.2	15.3	31.5	216	31
24	9.82	13.6	15.5	30.9	168	52
25	17.73	11.3	14.5	34.0	249	30
26	18.48	12.1	13.3	34.6	180	31
27	19.00	11.4	13.3	32.1	194	39
28	14.03	14.0	15.4	37.0	194	24
29	10.29	14.0	14.5	34.1	157	43
30	15.98	12.8	14.4	31.0	182	49
31	10.61	13.4	14.0	31.9	225	29
32	17.32	11.8	9.7	33.5	210	29
33	15.51	12.8	13.5	37.4	237	43
34	18.83	12.3	12.9	26.6	202	30
35	18.06	13.4	12.4	27.4	211	28
36	21.18	11.3	12.4	32.0	209	26
37	17.63	12.1	12.7	35.0	196	32
38	16.75	13.4	15.2	40.0	153	34
39	19.17	11.9	14.5	33.1	224	25
40	17.50	11.4	14.4	31.8	109	29
41	16.82	12.4	14.2	33.7	171	30
42	19.82	16.6	16.7	32.4	201	29
43	17.92	12.8	17.0	33.8	211	35
44	20.20	11.7	11.7	42.3	210	48
45	18.40	13.0	13.4	32.0	192	32
46	17.60	13.5	20.4	45.0	223	40
47	14.69	15.7	23.0	43.5	150	46
48	17.86	11.3	14.9	25.8	222	27
49	19.01	10.8	14.3	40.4	211	47
50	17.07	13.9	15.5	37.0	218	30

APPENDIX I (continued)

A	B	C	D	E	F	M
51	18.15	10.8	14.0	36.3	210	26
52	19.58	15.8	13.5	33.7	174	29
53	19.11	12.5	11.7	27.8	202	34
54	18.65	11.3	15.3	32.9	222	30
55	18.03	13.6	18.5	33.8	180	32
56	18.55	12.0	14.5	33.2	165	33
57	18.08	14.2	17.3	28.2	152	29
58	19.83	14.6	14.2	32.3	206	28
59	18.33	14.6	14.4	27.6	215	27
60	18.27	13.1	13.9	33.6	153	45
61	16.80	13.7	14.0	36.6	185	61
62	16.52	12.7	15.5	27.0	234	35
63	18.67	11.7	12.8	29.0	212	29
64	15.33	12.3	16.5	33.6	190	46
65	18.70	13.8	15.1	36.4	157	23
66	19.84	14.0	15.1	31.3	226	32
67	16.96	11.1	14.8	32.5	203	35
68	18.21	12.1	14.7	33.8	200	30
69	18.91	13.5	16.0	30.4	206	39
70	17.76	11.9	14.8	33.9	201	29
71	18.15	14.3	14.3	33.3	168	31
72	15.34	13.8	13.6	37.0	189	34
73	17.30	14.8	20.5	38.0	180	34
74	18.27	13.9	13.5	27.3	244	31
75	15.00	15.0	19.0	35.4	184	48
76	16.72	12.0	14.0	34.8	191	31
77	18.69	13.5	14.0	35.0	209	28
78	17.96	13.4	15.1	31.0	189	33
79	17.07	10.4	13.4	36.2	202	40
80	20.60	14.4	19.5	41.8	193	29
81	17.41	13.8	13.7	32.6	207	27
82	10.30	12.9	14.7	33.7	144	47
83	17.59	13.6	14.8	30.1	221	31
84	18.85	13.1	16.0	35.5	192	40
85	18.33	11.5	12.5	36.5	204	53
86	18.12	13.5	12.5	26.5	203	28
87	15.95	14.4	19.3	40.9	169	27
88	18.40	12.6	15.5	47.3	175	53
89	19.00	13.3	14.8	29.9	211	30
90	18.33	15.7	19.3	31.4	178	31
91	17.93	12.1	14.4	33.3	151	24
92	17.08	12.3	16.6	30.1	201	24
93	17.77	12.9	13.8	31.0	169	30
94	15.60	14.4	15.8	28.4	220	26
95	19.55	12.7	13.7	30.9	168	42
96	15.65	12.3	15.1	30.4	236	31
97	18.41	14.1	18.4	42.8	172	29
98	19.31	13.2	16.0	35.3	163	30
99	16.00	14.7	14.1	34.7	182	24
100	18.27	10.9	12.6	33.4	217	29

APPENDIX I (continued)

A	B	C	D	E	F	M
101	18.28	10.2	13.0	27.1	218	29
102	19.20	16.4	14.4	35.7	218	27
103	16.90	9.9	14.2	27.1	191	23
104	16.04	11.1	12.8	36.5	223	25
105	18.70	12.0	16.2	33.2	211	26
106	17.90	13.0	13.7	30.5	192	32
107	15.21	13.8	15.9	34.7	194	48
108	19.05	13.9	14.4	31.0	194	34
109	19.07	11.9	15.1	37.8	157	24
110	20.00	14.7	13.4	38.8	213	27
111	18.30	12.4	12.5	32.6	203	23
112	17.59	17.0	18.3	49.5	187	40
113	17.59	11.1	13.8	35.0	193	31
114	19.61	14.4	17.2	38.3	195	29
115	19.00	13.8	13.6	38.2	164	37
116	14.40	15.3	13.7	34.3	171	47
117	18.12	12.1	15.6	36.4	264	35
118	19.44	14.1	14.7	31.5	223	60
119	17.17	16.9	21.9	43.0	174	50
120	17.41	13.6	16.2	33.9	192	39
121	17.32	12.5	15.2	35.6	180	22
122	18.39	8.9	12.2	31.1	219	35
123	18.71	14.4	16.1	32.6	214	42
124	18.10	12.5	13.1	27.8	192	28
125	17.04	13.5	14.6	36.0	166	42
126	18.39	14.8	15.5	34.5	207	31
127	18.03	13.5	16.0	36.0	203	30
128	19.25	14.9	12.8	28.5	197	32
129	19.13	11.6	12.6	27.1	189	37
130	19.14	11.7	12.7	42.9	198	38
131	17.07	17.8	13.2	29.5	182	37
132	19.63	12.4	15.2	29.3	182	29
133	16.07	12.0	17.1	39.8	134	52
134	14.10	21.5	15.9	40.9	197	57
135	18.75	13.6	17.8	39.4	150	35
136	19.17	12.0	12.8	28.4	221	31
137	19.64	11.8	15.8	40.4	238	26
138	18.84	13.6	14.8	36.6	242	28
139	19.37	13.3	11.9	30.4	252	26
140	18.57	14.3	15.0	35.1	165	27
Mean	17.50	13.1	14.9	34.2	199.2	33.0
Standard deviation (number 140)	1.65	1.86	1.99	4.67	24.83	
Means corrected for Ewald chronoscope (see Fig. 4)		.241 sec.	.274 sec.	.629 sec.		

The time for the outdoor driving is given in minutes, the braking time, foot-reacting time and simple reacting time in hundredths of a second; the foot movement in number of taps in 2 minutes.

A = Subject.
B = Experimental driving course scores.
C = Simple reaction time.
D = Foot reaction time.
E = Foot braking time.
F = Speed-of-foot movement.
M = Age.

APPENDIX II

TEST SCORES OF IOWA STATE COLLEGE UNIT

A	B	C	D	E	F	G	H	I	J	K	L	M
1	18.0	.218	.248	.477	200	.407	.219	.223	76.2	46.1	RL*	20
2	12.4	.274	.267	.845	205	.483	.264	.297	64.7	41.9	RL	28
3	14.1	.268	.259	.890	178	.476	.290	.289	62.1	38.7	RL	25
4	16.9	.226	.256	.492	204	.418	.229	.249	81.2	40.2	RL	23
5	16.5	.211	.262	.573	219	.421	.223	.222	80.0	40.0	RL	20
6	13.3	.242	.232	.470	189	.450	.230	.284	153.2	38.3	R	22
7	13.0	.243	.256	.497	145	.440	.243	.214	78.6	40.6	RL	18
8	13.8	.264	.300	.888	177	.453	.235	.238	102.0	45.0	R	22
9	13.2	.174	.262	.482	204	.376	.182	.213	62.8	34.7	RL	19
10	15.4	.169	.228	.512	204	.401	.175	.192	65.2	34.4	RL	21
11	16.5	.210	.245	.918	188	.440	.238	.247	72.9	37.6	RL	27
12	12.5	.242	.238	.694	209	.410	.253	.243	73.6	39.1	RL	25
13	13.0	.214	.265	.698	203	.456	.225	.248	106.3	54.4	RL	21
14	17.2	.248	.264	.590	236	.430	.236	.261	75.0	37.7	RL	27
15	14.0	.240	.284	.762	171	.426	.251	.263	74.1	45.7	RL	18
16	17.1	.206	.231	.581	212	.410	.217	.216	71.0	38.1	RL	23
17	16.3	.230	.288	.487	207	.440	.259	.235	83.5	39.3	RL	22
18	15.0	.240	.261	.689	188	.440	.240	.256	89.1	44.1	RL	19
19	16.5	.288	.247	.639	218	.422	.256	.256	57.8	40.2	RL	17
20	17.7	.190	.231	.481	207	.392	.201	.201	77.1	32.1	RL	..
21	18.4	.217	.224	.502	182	.401	.222	.218	98.2	34.5	R	20
22	14.8	.229	.264	.859	195	.405	.229	.240	77.0	37.0	L	..
23	15.0	.256	.260	.764	162	.442	.249	.259	85.3	33.1	R	15
24	13.7	.207	.254	1.148	145	.425	.278	.326	100.0	29.6	L	52
25	17.1	.201	.237	.491	209	.402	.217	.221	61.2	31.6	RL	18
26	16.7	.218	.241	.592	202	.416	.221	.220	71.4	36.2	RL	21
27	16.8	.204	.250	.670	197	.421	.230	.241	80.2	38.1	RL	..
28	17.0	.211	.239	.601	206	.418	.215	.240	77.2	36.2	RL	23
29	16.5	.235	.242	.591	202	.427	.240	.233	71.1	35.1	RL	..
30	14.3	.238	.258	.672	189	.431	.242	.248	75.2	36.3	RL	21
31	16.0	.231	.240	.491	197	.417	.230	.245	72.1	40.2	RL	20
32	14.7	.231	.240	.667	153	.471	.215	.242	75.5	40.0	RL	..
33	12.5	.247	.238	.694	209	.410	.253	.243	73.6	39.1	RL	25
34	15.4	.256	.286	.660	194	.429	.262	.281	73.3	59.9	RL	18
35	17.1	.214	.229	.482	212	.412	.220	.218	64.1	34.8	RL	20
36	14.7	.250	.258	.720	192	.452	.261	.260	82.1	40.2	RL	20
37	15.4	.246	.271	.721	184	.451	.256	.261	91.2	42.1	RL	22
38	12.1	.261	.263	.716	192	.451	.260	.264	82.2	45.6	RL	..
Means	15.3	.2535	.2535	.6489	193.9	.4291	.2363	.2453	79.4*	39.9*		
Standard deviation	1.77	.0159	.0174	.0712	19.0	.0239	.0234	.0284	16.4	6.3		
Correction for Ewald chronoscope									1.461	.734 sec.		

* RL—Neither eye is dominant.

A = Subject.
B = Experimental driving course scores.
C = Simple reaction time.
D = Foot reaction time.
E = Foot braking time.
F = Speed-of-foot movement.
G = Choice reaction time.
H = Reaction time after pursuitmeter test.
I = Simple reaction time with high frequency sound present.
J = Accommodation to light.
K = Accommodation to darkness.
L = Ocular dominance.
M = Age.

APPENDIX II

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APPENDIX III

IOWA STATE COLLEGE RATING SCALE FOR AUTOMOBILE DRIVERS

J. E. Evans and LeVerne Johnson

Ames, Iowa

Name .... Date .... 193....... Score..........

Make of Car ....Model: Sedan, coach, touring, coupe, truck. 193....

Time of starting: ....; Time of finishing: ....; Total time: ....

A. STARTING:						
1. Fails to look around before starting	0	5	10	15	20	25
2. Stays too long in low or second	0	5	10	15	20	25
3. Unnecessarily fast getaway	0	5	10	15	20	25
4. Kills engine	0	5	10	15	20	25
5. Races engine	0	5	10	15	20	25
6. Fails to start smoothly	0	5	10	15	20	25
7. Attempts to start with brake set	0	5	10	15	20	25
8. Starts under too much power; rear wheels slip	0	5	10	15	20	25
B. STOPPING:						
1. Fails to signal or to give proper signal for stop	0	5	10	15	20	25
2. Slows down too suddenly	0	5	10	15	20	25
3. Fails to set emergency brake when necessary	0	5	10	15	20	25
4. Jerky stop	0	5	10	15	20	25
5. Stops where he endangers traffic	0	5	10	15	20	25
C. TURNING:						
1. Fails to get into proper lane for turn	0	5	10	15	20	25
2. Fails to signal or gives improper signal for turn	0	5	10	15	20	25
3. Fails to look in mirror or out window	0	5	10	15	20	25
4. Swings wide on the corner	0	5	10	15	20	25
5. Cuts too closely on the corner	0	5	10	15	20	25
6. Turns with too much speed	0	5	10	15	20	25
D. BACKING:						
1. Fails to look behind before backing	0	5	10	15	20	25
2. Steering uncertain; lack of skill	0	5	10	15	20	25
3. Lacks confidence	0	5	10	15	20	25
E. PARKING:						
1. Bumps other cars or obstacles	0	5	10	15	20	25
2. Climbs curb or scours tires	0	5	10	15	20	25
3. Parks too far from curb	0	5	10	15	20	25
4. Clumsy; no skill	0	5	10	15	20	25
5. Slow	0	5	10	15	20	25
F. PASSING:						
1. Does not await clear distance ahead	0	5	10	15	20	25
2. Too close before turning out	0	5	10	15	20	25
3. Fails to use horn properly	0	5	10	15	20	25
4. Cuts in too quickly after passing	0	5	10	15	20	25
5. Too slow in passing	0	5	10	15	20	25
6. Poor judge of speed of car passed	0	5	10	15	20	25
7. Poor judge of speed of approaching car	0	5	10	15	20	25
8. Poor judge of speed of his own car	0	5	10	15	20	25

G. SIGNS:						
1. Fails to notice signs	0	5	10	15	20	25
2. Fails to stop for stop sign	0	5	10	15	20	25
3. Fails to observe warning signs	0	5	10	15	20	25
4. Observation of signs poor	0	5	10	15	20	25
H. HILLS:						
1. Cannot stop and start without backing	0	5	10	15	20	25
2. Poor shifting of gears	0	5	10	15	20	25
3. Foot-work poor	0	5	10	15	20	25
I. SPEED:						
1. Speed greater than ability warrants	0	5	10	15	20	25
2. Speed greater than traffic warrants	0	5	10	15	20	25
3. Speed greater than highway warrants	0	5	10	15	20	25
4. Speed too slow for conditions	0	5	10	15	20	25
5. Poor judge of distance	0	5	10	15	20	25
6. "Brakes" excessively	0	5	10	15	20	25
7. Speed erratic	0	5	10	15	20	25
8. Takes unnecessary chances	0	5	10	15	20	25
J. ATTITUDE TOWARDS OTHERS:						
1. Depends too much on others for safety	0	5	10	15	20	25
2. Inconsiderate toward pedestrians	0	5	10	15	20	25
K. MISCELLANEOUS:						
1. Uses horn properly	0	5	10	15	20	25
2. Fails to keep in right lane on straightaway	0	5	10	15	20	25
3. Drives defensively	0	5	10	15	20	25
4. Follows too closely	0	5	10	15	20	25
5. Stays in proper lane at corners, on curves, and at top of hills	0	5	10	15	20	25
6. Runs off shoulder or curb	0	5	10	15	20	25
7. Posture poor; grip on wheel too tight	0	5	10	15	20	25
8. Relaxed but alert	0	5	10	15	20	25
9. Indifferent to conditions ahead	0	5	10	15	20	25
10. Right front wheel not located	0	5	10	15	20	25
11. Easily disturbed emotionally	0	5	10	15	20	25
12. Can be distracted easily; sees too much	0	5	10	15	20	25
13. Too tense	0	5	10	15	20	25
14. Considers rights of pedestrians	0	5	10	15	20	25
15. Self-confident	0	5	10	15	20	25
16. Driver aware of all influences which may relate to his driving	0	5	10	15	20	25
17. Localizes sounds well	0	5	10	15	20	25
18. In night driving uses lights courteously	0	5	10	15	20	25

REMARKS:

SCORING:

$$\text{Average score} = \frac{\text{sum of } \surd\text{-ed numbers}}{\text{number of } \surd\text{s}} = \ldots\ldots\ldots\ldots .$$

(The "best" drivers receive the highest scores.)

Examiner...

EXAMINATION OF BUTTER WITH THE BURRI SMEAR CULTURE TECHNIC[1]

H. F. LONG AND B. W. HAMMER

From the Dairy Industry Section, Iowa Agricultural Experiment Station

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From a bacteriological standpoint butter differs strikingly from such products as milk or cream in which bacteria can migrate readily because water is the continuous phase. In butter the water is present as small droplets surrounded by fat. Presumably, the bacteria in butter are largely contained in these droplets and are held at certain points, many of the droplets being sterile since butter normally contains from 10 to 18 billion per gram (3) while the bacterial content is always much lower than this. Since growth of bacteria in butter occurs primarily in infected droplets, the distribution of the bacteria may be very irregular, both as to numbers and species.

In the plate method of studying butter bacteriologically a relatively large portion is shaken in a water blank. This procedure has a decided disadvantage from the standpoint of showing the distribution of organisms in the sample. Because of this objection the Burri smear culture technic (1) has been adapted to the examination of butter at the Iowa Agricultural Experiment Station in an attempt to obtain information not provided by the usual plating procedure.

METHOD

The method consists of picking small portions of butter with a platinum needle and spreading each portion on the surface of a dry agar slope.

The butter to be examined is placed in a sterile petri dish and brought to approximately 21° C.; at this temperature it has a reasonably firm body and small amounts can be picked easily. The butter may come from a surface that has been exposed for some time, a freshly cut surface, or a freshly broken surface. Under a binocular giving approximately a 6X magnification very small amounts are picked with a flamed platinum needle. The use of a binocular is advisable since it aids in keeping the portions of butter uniform in size and also in actually picking each portion rather than scraping it from a relatively large area; the latter point is important in obtaining information on the distribution of organisms. A needle of platinum, rather than some other material, is used because of the greater resistance to changes during heating. Each portion of butter is spread on the surface of a dry agar slope, care being taken to distribute it evenly. Ordinarily 25 portions of butter are picked from a sample. Larger or smaller numbers may be used if either more or less detail in the results is desired.

The slopes are incubated at 21° C. for 4 to 5 days and then counted. Other incubation conditions can be used for special purposes but temperatures appreciably higher than 21° C. prevent the growth of certain organ-

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isms important in butter while temperatures appreciably lower often result in slow growth. The maximum number of colonies that can be counted satisfactorily on a slope is approximately 100, depending somewhat on the size of the colonies and the tendency for them to grow together. In some instances it is an advantage to examine the tubes showing extensive growth before the usual incubation period has expired. Numbers exceeding 100 per tube often can be estimated with considerable accuracy, but under these conditions it is probable that many organisms fail to grow.

The amount of butter picked was estimated in a number of trials by determining the collective weight of 15 portions. The results indicated that each portion weighed approximately 0.05 mg., or 1/20,000 gram. The weight of 15 portions remained fairly constant if the consistency of the butter was the same and if the needles used to pick the butter were clean. With temperatures appreciably higher than 21° C. there was a tendency to pick larger amounts of butter. By multiplying the average number of colonies per slope by 20,000 the results are obtained on a gram basis. There are various factors which tend to make counts obtained with this method lower than counts obtained with the plate method, as is pointed out later.

Beef infusion agar (pH 6.8) has been employed frequently with the method because this medium is so generally satisfactory for the examination of butter. However, for special purposes other media may be used. In studying the distribution of butter culture organisms tomato agar would be better than beef infusion agar. For the detection of proteolytic and lipolytic bacteria, plates may be prepared with agar containing milk, or emulsified fat, or both (2). A number of portions of butter can be smeared on a plate by marking off sections for inoculation. When plates are employed air contamination is a greater factor than when tubes are used. Regardless of the medium or container, it is essential that the agar surface be dry so that the colonies develop independently.

EXPERIMENTAL

The Burri smear culture technic has been used to examine many samples of commercial and experimental butter, some of which were normal and some abnormal. The commercial butter was from various points in Iowa and surrounding states. Commonly 25 slopes were prepared from a commercial sample and 10 from an experimental sample; plate counts also were usually made, using beef infusion agar (pH 6.8) and incubating 4 days at 21° C.

NORMAL, COMMERCIAL, SALTED BUTTER

The results on 10 samples are presented in table 1. The data show great differences in the numbers of organisms in the samples and striking variations in the distribution of bacteria in each sample containing many organisms. A variation from 0 to 176 colonies on a slope occurred with sample 6 and from 6 to 140 with sample 8; conspicuous irregularities also occurred with samples 1, 2, 3, 7, 9 and 10. Some of the slopes inoculated from samples 4 and 5 showed no colonies while the maximum number was 3.

The slopes from a sample frequently showed variations in colony

TABLE 1. *Results with the Burri technic on normal, commercial, salted butter*
Numbers of colonies on slopes incubated 4 days at 21° C.

Slope number	Butter number									
	1	2	3	4	5	6	7	8	9	10
1	17	22	13	0	1	1	22	88	20	3
2	39	8	5	0	0	2	36	35	14	0
3	77	9	10	0	1	0	37	45	12	1
4	7	12	1	0	0	2	14	9	23	6
5	51	36	5	0	0	6	6	58	36	0
6	29	49	11	3	0	28	25	6	20	3
7	24	21	23	1	0	7	12	28	42	0
8	27	8	11	1	0	2	55	74	21	2
9	88	9	10	2	1	1	14	6	21	4
10	29	6	6	2	0	2	51	70	23	4
11	25	11	3	0	1	1	15	32	25	2
12	15	8	2	0	0	3	26	47	13	0
13	18	13	7	0	1	1	10	48	28	11
14	97	14	38	1	0	176	40	50	34	6
15	49	11	5	2	1	4	39	62	7	5
16	28	15	5	0	0	3	26	48	11	5
17	50	11	3	0	0	19	19	59	6	1
18	33	44	3	0	0	2	50	17	9	1
19	27	7	3	2	2	5	28	17	15	8
20	42	46	1	1	0	17	33	34	80	1
21	24	56	7	2	0	3	26	12	23	4
22	9	9	5	0	3	1	30	23	23	7
23	62	20	6	0	2	15	4	33	30	6
24	69	8	4	1	0	1	32	10	16	2
25	60	2	1	3	0	3	28	140	13	0

types. Often there was a predominant type which occurred in practically all the tubes while some tubes contained other organisms and some did not. The types developing on slopes were the same as those developing on plates (prepared by the dilution method) with samples 2, 3, 4, 5, 6 and 7. With samples 1 and 9 micrococci developed on the slopes but not on plates suitable for examination. Sample 8 gave butter culture type colonies on the slopes but not on the plates, while with sample 10 micrococci and large spreading white colonies developed on the slopes but not on plates.

With the Burri technic all the colonies are on the surface of the agar and thus are more easily differentiated into colony types than with the plating technic in which many colonies are subsurface.

ABNORMAL, COMMERCIAL, SALTED BUTTER

Data on 6 samples are given in table 2. The numbers of colonies were much larger than with the normal butter and frequently the growth on a slope was so heavy that the total number of colonies could not be estimated. Variations in distribution are again evident and certain samples gave relatively little growth on some slopes and extremely heavy growth on others. With sample 4 one slope contained only 9 colonies while another was overgrown, and with sample 5 one slope contained only 6 colonies while some were overgrown.

TABLE 2. *Results with the Burri technic on abnormal, commercial, salted butter*
Numbers of colonies on slopes incubated 4 days at 21° C.

Slope number	Butter number					
	1	2	3	4	5	6
1	o.g.	180	330	108	200	384
2	290	120	240	52	456	620
3	768	700	700	14	200	o.g.
4	480	600	210	32	410	1824
5	1080	180	660	9	378	o.g.
6	300	370	600	20	128	910
7	o.g.	250	328	126	112	384
8	325	220	368	730	378	460
9	o.g.	370	540	252	64	o.g.
10	90	240	120	276	140	410
11	70	540	350	360	6	700
12	o.g.	200	300	640	o.g.	o.g.
13	50	310	240	632	240	o.g.
14	o.g.	80	760	380	50	456
15	468	340	800	94	o.g.	375
16	420	280	280	130	62	o.g.
17	400	105	o. g.	574	450	755
18	575	120	360	o.g.	200	660
19	100	430	290	720	324	o.g.
20	o.g.	400	108	624	350	o.g.
21	1680	460	250	384	o.g.	o.g.
22	o.g.	600	370	960	462	o.g.
23	98	370	340	252	550	560
24	84	310	650	108	276	320
25	61	600	1920	200	192	325

Note: o.g. = overgrown.

The colony types developing on slopes and plates from the same butter were identical with 3 samples (2, 4 and 5). The slopes from sample 1 contained yellow and white micrococci, a few fluorescent organisms and a few spore formers in addition to numerous smooth white colonies, while the plates contained only the latter type. Slopes from sample 3 contained a large number of very small, white colonies consisting of gram-positive rods; this species failed to develop on plates suitable for examination. The colony types on slopes and plates from sample 6 were the same except that a few fluorescent organisms were found on the slopes but not on the plates.

COMMERICAL, UNSALTED BUTTER

Table 3 presents results on 8 samples. With this type of butter the cream is often ripened to a high acidity so that butter culture organisms are very numerous in the finished product; because of this a separate count was made of the colonies which resembled butter culture types. The butter was normal with the exception of sample 6, which was cheesy.

Butter culture organisms predominated in samples 1, 2, 3, 4 and 5; there was considerable variation in the colonies per slope although the counts were regularly high. All the colonies on the slopes from sample 1 suggested butter culture organisms, but a small number of other colony

TABLE 3. *Results with the Burri technic on commercial, unsalted butter*
Numbers of colonies on slopes incubated 4 days at 21° C.

Slope number	Butter number 1: probably b.c.* types	1: not b.c.* types	2: probably b.c.* types	2: not b.c.* types	3: probably b.c.* types	3: not b.c.* types	4: probably b.c.* types	4: not b.c.* types	5: probably b.c.* types	5: not b.c.* types	6†: probably b.c.* types	6†: not b.c.* types	7: probably b.c.* types	7: not b.c.* types	8: probably b.c.* types	8: not b.c.* types
1	600	0	1,056	2	o.g.	3	329	0	756	1	0	o.g.	0	154	0	2
2	800	0	350	2	880	2	1,548	0	480	2	0	19	0	2	0	6
3	3,600	0	910	0	672	2	858	0	880	0	0	16	0	1	0	2
4	2,000	0	568	1	180	2	1,700	0	2,240	0	0	660	0	7	0	3
5	o.g.	0	1,584	2	660	6	1,140	2	612	0	0	26	0	9	0	16
6	880	0	o.g.	2	420	2	800	1	240	0	0	26	0	13	0	2
7	1,100	0	960	2	530	1	1,072	2	728	2	0	o.g.	0	14	0	0
8	1,600	0	704	0	322	1	1,900	5	1,000	3	0	118	0	1	0	0
9	600	0	350	2	260	1	840	1	250	0	0	138	0	16	0	3
10	450	0	770	0	378	2	456	6	882	0	0	2	0	188	0	2
11	o.g.	0	320	1	448	3	2,250	0	1,200	3	0	600	0	3	0	0
12	1,400	0	576	2	132	3	608	1	225	2	0	6	0	1	0	63
13	480	0	1,040	0	240	6	846	0	588	7	0	61	0	0	0	2
14	468	0	336	5	400	1	1,120	0	644	9	0	1	0	7	0	5
15	600	0	608	2	330	1	1,800	0	2,200	0	0	o.g.	0	8	0	2
16	o.g.	0	450	1	126	2	1,400	0	280	6	0	6	0	5	0	1
17	688	0	1,500	0	636	1	650	0	672	2	0	12	0	0	0	1
18	928	0	500	3	324	1	720	0	476	0	0	7	0	5	0	2
19	700	0	2,000	2	434	3	750	0	840	0	0	18	0	5	0	4
20	1,300	0	352	0	532	1	500	0	480	0	0	144	0	0	0	3
21	2,000	0	210	1	924	2	800	2	372	0	0	8	0	1	0	3
22	2,500	0	546	2	588	2	672	0	210	0	0	94	0	4	0	2
23	636	0	460	6	238	1	616	2	2,500	0	0	o.g.	0	5	0	1
24	760	0	588	0	370	0	200	1	768	2	0	23	0	o.g.	0	1
25	1,080	0	1,240	1	660	0	936	5	736	1	0	4	0	96	0	0

Note: * b.c. = butter culture. † Butter was cheesy. o.g. = over grown.

types developed on the slopes from sample 2, 3, 4 and 5. No butter culture type colonies were noted on slopes from samples 6, 7 and 8. The numbers of colonies on the slopes from samples 6 and 7 were extremely variable, ranging from 0 to numbers impossible to estimate. With sample 8 the numbers also were rather irregular.

FRESHLY CHURNED, EXPERIMENTAL, UNSALTED BUTTER

The results on 18 samples are presented in table 4; the butter was made from pasteurized cream inoculated with pure cultures of bacteria. The slopes picked from most of the samples were either sterile or contained relatively few colonies. With the samples containing larger numbers of organisms, the colonies per slope varied from 4 to 26, from 9 to 25, and from 6 to 35.

EXPERIMENTAL, UNSALTED BUTTER HELD 4 DAYS AT 21°C.

The 18 samples of butter examined when fresh were held 4 days at 21° C. and re-examined; table 5 presents the results. The number of colonies on many slopes was high, as would be expected since there was opportunity for extensive development owing to the favorable temperature and lack of salt. Particularly striking variations in the distribution of organisms were noted with samples 4, 13 and 14 in each of which one slope showed no growth while one or more slopes was overgrown; in the initial examination of these samples 8 slopes picked from sample 4, and 9 from each of samples 13 and 14 were sterile. Most of the slopes picked from samples 6 and 15 were sterile although in each case 2 slopes contained a relatively large number of colonies; originally only one slope picked from sample 6 and none from sample 15 showed growth.

COMPARISON OF COUNTS BY THE BURRI TECHNIC AND THE PLATE METHOD

Counts obtained by multiplying the average number of colonies per slope by 20,000 did not agree closely with plate counts and often were considerably lower. In general the results of the two methods agreed better on low count samples than on high count samples. In the Burri technic, clumps are not broken up as they may be when shaken in a

TABLE 4. *Results with the Burri technic on freshly churned, experimental, unsalted butter*

Numbers of colonies on slopes incubated 4 days at 21° C.

Slope number	Butter number																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16*	17*	18*
1	0	0	0	0	0	0	1	1	0	8	22	32	0	0	0	3	1	1
2	0	0	0	0	0	0	1	0	0	16	9	10	0	0	0	3	2	0
3	0	0	0	1	0	0	2	1	0	15	21	11	3	1	0	2	2	3
4	0	0	0	0	0	1	0	0	1	5	25	18	0	0	0	4	1	1
5	0	0	0	0	0	0	0	0	0	11	16	26	0	0	0	0	2	0
6	0	0	0	1	2	0	0	1	0	26	13	35	0	0	0	2	0	0
7	0	0	0	0	0	0	0	0	0	9	16	13	0	0	0	1	3	1
8	1	0	0	0	0	0	0	0	0	9	23	6	0	0	0	1	1	0
9	0	0	0	0	0	0	0	0	0	16	14	16	0	0	0	2	0	1
10	0	0	0	0	0	0	0	0	0	4	15	21	0	0	0	3	3	0

Note: * Cream inoculated with a yeast.

TABLE 5. *Results with the Burri technic on experimental, unsalted, butter held 4 days at 21° C.*

Numbers of colonies on slopes incubated 4 days at 21° C.

Slope number	Butter number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16*	17*	18*
1	0	0	7	0	0	0	840	0	11	4,000	420	560	140	448	0	13	60	31
2	700	0	0	2,200	0	0	60	2	62	770	o.g.	432	250	o.g.	3	11	16	9
3	4	0	6	o.g.	0	0	600	3	192	816	336	528	570	504	0	5	12	30
4	7	0	80	336	0	0	21	994	14	600	2,128	384	0	20	0	37	22	12
5	1	0	47	2,500	0	65	92	2,200	86	2,240	952	184	352	2	0	10	11	30
6	600	22	0	4,500	0	142	204	2	1,218	1,400	288	288	560	22	0	16	21	120
7	63	0	0	30	0	0	21	434	40	o.g.	1,440	216	o.g.	480	0	29	27	14
8	1	0	36	o.g.	0	3	73	81	686	2,000	714	340	o.g.	156	1	40	o.g.	17
9	0	7	0	o.g.	1	0	91	90	19	o.g.	1,200	82	560	0	39	26	56	19
10	0	0	20	o.g.	0	0	350	0	0	1,330	o.g.	1,248	o.g.	176	304	34	45	26

Note: * Cream inoculated with a yeast. o.g. = overgrown.

water blank. If large numbers of organisms are present in butter the slopes are certain to be overcrowded so that some organisms do not grow; moreover, detailed counting is difficult. The development of certain colony types on slopes that do not develop on plates causes variations in the other direction. The picking of 25 portions, rather than 10, commonly gave a somewhat closer agreement in the results of the two methods, presumably because of a more accurate average number per slope.

DISCUSSION OF RESULTS

The Burri technic is useful for the examination of butter because it gives information on the distribution of bacteria, both as to numbers and species. The results show that the distribution of bacteria in both normal and abnormal butter is often highly variable. Growth appears to be largely limited to certain points, presumably infected moisture droplets. The final distribution of organisms may be influenced by such factors as irregularities in salt content of various moisture droplets and in the size of the droplets, as well as in the original contamination.

The better differentiation of colonies on slopes is a distinct advantage from the standpoint of studying irregularities in distribution of colony types and in isolating the various species. The presence of certain colony types on slopes but not on plates from a sample suggests that the technic may be especially valuable for studying microbiological defects of butter. With the technic, clumps are not broken up as they are in the plate method and a number of organisms of a species may be left at one point on a slope; under these conditions, growth might take place when it would not if the organisms were well distributed in a water blank and then plated. In certain instances a species present in a sample in small numbers as compared to the total numbers might be diluted out in plates suitable for examination and yet appear on Burri slopes.

The failure of Burri counts to agree with plate counts is not a serious disadvantage since the technic readily distinguishes low count and high count butter. Ordinarily total counts on butter are not significant from a bacteriological standpoint since often a large percentage of the flora is made up of butter culture organisms. The species present in a sample of butter appear to be more important than total numbers; since the Burri technic gives a good differentiation of colony types and may even yield growth of species that do not develop on plates it is especially useful.

SUMMARY

Butter was examined bacteriologically with an adaptation of the Burri smear culture technic which consists of picking small amounts with a needle and smearing each on the surface of a dry agar slope; approximately 1/20,000-gram portions were satisfactory. By means of the method the bacteria in various types of butter were often found to be very irregularly distributed, both from the standpoint of numbers and colony types. The colonies on the slopes were well differentiated since they all developed on the surface of the agar. In some instances colony types were found on slopes which were not evident on plates.

PLATE I

Variations in the numbers and types of colonies on slopes, each inoculated with approximately a 1/20,000 gram portion of a churning of butter.

PLATE I

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A LOGARITHMIC SECTOR AND A SLIT FOR USE IN SPECTROGRAPHIC ANALYSIS

HARLEY A. WILHELM

From the Department of Chemistry, Iowa State College

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The sector described by Twyman and Simeon[1] for use in quantitative spectrographic analysis has a single logarithmic spiral cut in a metal disc. If such a sector is rotated before the slit of a stigmatic spectrograph during an exposure, the lines that are produced in the spectrogram decrease in density toward one end. The greater the intensity of a spectrum line that is radiated from the light source, the greater the length of the line in the spectrogram. Under conditions of controlled excitation, the intensity of a spectrum due to a small amount of an element is generally proportional to the amount of the element in the light source. Therefore, measurements on lengths of lines give the data for quantitative determinations by this method. For example, Plate 1, fig. 1 shows the results of exposures that have been made from a series of standards for analysis of fluorine in samples of bone flour. The calcium fluoride band at wavelength 5291.Å increases in length as the fluorine in the standards increases from 0.01 per cent to 0.20 per cent. In making an analysis, the unknown sample is exposed in the same manner as the standards and a comparison of line lengths gives the percentage fluorine in the sample. The error in a determination by this method is usually less than one-tenth of the total fluorine in the sample.

The sector that is described here has two logarithmic spirals, one in each of the two halves of the disc as shown in fig. 1. The equation for each of these spirals in this double spiral sector is

$$-\log \phi = 0.2\ l$$

Where ϕ represents the fractional opening at a distance l millimeters measured radially inward from the outermost part of the curve. Since ϕ for each spiral is expressed as a fraction of the half circle, $\phi/2 = \theta$, where θ is the fraction of a complete circle. Substitution of 2θ for ϕ in the above equation for the spirals gives

$$-\log \theta = 0.301 + 0.2\ l$$

which is the equation of the single spiral sector. Therefore, the quantitative relationships that hold for the single spiral sector (1) hold also for each spiral of the double sector. The double spiral sector, however, requires a total exposure time that is only half as great as that with the single spiral sector in order to give the same effective exposure. Furthermore, the double spiral sector is self counter-balanced.

A template for cutting spirals in half discs has been made by the College Instrument Shop. Two half discs, each having a logarithmic spiral, are mounted permanently on a faceplate to give a disc having the periphery that is shown in fig. 1. The dimensions correspond to a double

[1] Twyman and Simeon, Trans. Optical Soc. (London), 31:169 (1929-30).

sector cut from a disc 5.5 inches in diameter. A double logarithmic spiral sector that was made for this laboratory shows a mean deviation of less than $\pm$ 0.02 millimeter from the theoretical curve for values of l between zero and eleven millimeters.

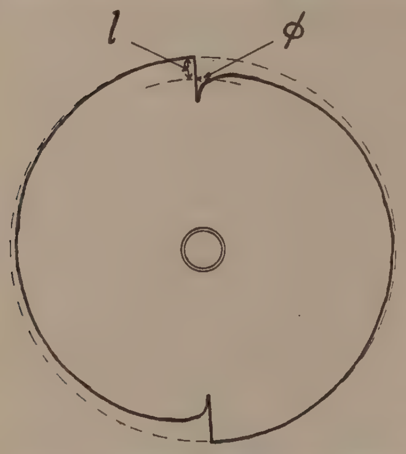


Fig 1. Double logarithmic spiral sector.

One of the conditions necessary for proper use of a logarithmic sector is that the plane of the spiral should be nearly coincident with the plane of the jaws of the slit. The ordinary non-symmetrical slit unit which is often used in qualitative spectrographic analysis is not applicable to work with the sector. A guideway for a sliding diaphragm and the slit adjustment drumhead stand out before the plane of the slit jaws and make it impossible to place the sector disc closer to the slit than about two millimeters. This type of slit can, however, be made to accommodate a logarithmic spiral sector in quantitative analysis by making a few alterations in the slit mechanism.

Plate I, fig. 2 shows a convertible slit that has been made from one of the common type slit units. The slit adjustment mechanism has been altered to bring the edge of the drumhead (A) flush with the jaws of the slit. This change involved placing a thick shim under the screw-block and using long pins in place of short ones in the slit jaw activating mechanism on the back side of the slit unit. The lower fixed guideway, for holding the Hartmann diaphragm (B) before the slit, was milled off and a detachable guideway (C) which can be mounted on the lower part of the slit unit was made for guiding the diaphragm into proper position. By removing the guideway (C) from the slit unit, the sector disc can be placed very near to the face of the jaws of the slit. The adjustable thin metal strip (D) covers the upper portion of slit that is not used with the sector.

The conversion of the slit from the form for qualitative work to the form suitable for use with the logarithmic spiral sector or *vice versa* can be made in a very few minutes.

PLATE I

Fig. 1. Standards for spectrographic determination of fluorine.
Fig. 2. Convertible slit unit.

PLATE I

Fig. 1

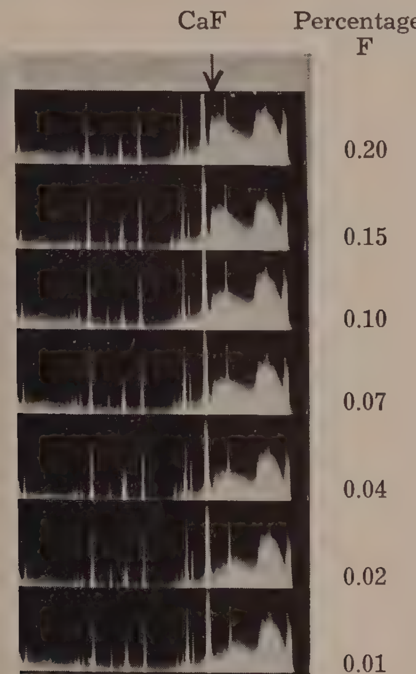
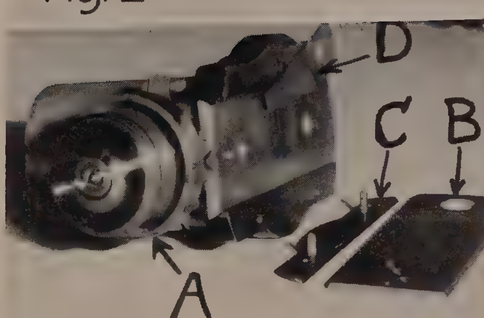


Fig. 2



EQUILIBRIUM OF A THIN PLATE, SYMMETRICALLY LOADED, ON A FLEXIBLE SUBGRADE*

D. L. HOLL

From the Department of Mathematics, Iowa State College

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In this note there is presented the derivation and the solution of an integro-differential equation associated with the following problem. A thin plate infinite in extent rests on a yielding subgrade or foundation. The plate is loaded by a normal surface loading on the upper surface and by the subgrade reaction pressure on the contacting surface. It is assumed that the whole system of loading, plate and subgrade, possesses axial symmetry and that the ordinary thin plate theory is applicable. The additional hypothesis is made that the plate maintains continuous contact with the subgrade without inducing any shearing resistance on the plate. A general result is obtained for the deflection of the plate for various types of loading and for subgrades of differing physical nature.

DERIVATION OF EQUATIONS

The plate equation in the axially symmetric case is

$$(1) \qquad N\nabla^4 w(r) = p(r) = p_0(r) - p_s(r),$$

where $w(r)$ is the axial deflection, $N = EI/(1-\nu^2)$ is the plate rigidity factor, and p_0 and p_s denote the surface loading of the plate and subgrade reaction, respectively.

Let $k(r,\theta;\varrho,\phi)$ denote the deflection at (r,θ) of the subgrade surface for a unit load applied on this surface at (ϱ,ϕ). This kernel or "influence function" is symmetrical in the variables and may be considered as known either by analytical considerations or by experimental determination since it depends upon the physical nature of the foundation itself. By reason of the symmetry involved, the deflection in any axial plane may be considered and θ may be taken equal to zero. Hence for an elementary load $p_s(\varrho)\,\varrho d\varrho d\phi$, the deflection w of the subgrade surface is

$$dw = k(r;\varrho,\phi)\,p_s(\varrho)\,\varrho d\varrho d\phi,$$

and for an elementary ring load between ϱ and $\varrho + d\varrho$ is

$$(2) \qquad dw = 2p_s(\varrho)\,\varrho d\varrho \int_0^{\pi} k(S)\,d\phi = 2\pi p_s(\varrho)\,d\varrho K(r,\varrho),$$

where

$$K(r,\varrho) = \int_0^{\infty} k(S)\,S dS \int_0^{\infty} u J_0(uS)\,J_0(ur)\,J_0(u\varrho)\,du$$

and $S^2 = r^2 + \varrho^2 - 2r\varrho\cos\phi$, and J_0 denotes the Bessel function of zero

* This paper is included under the title "Thin Plates on Elastic Foundations" submitted January, 1938, for presentation at the Fifth International Congress for Applied Mechanics in Boston, September, 1938.

order. Hence the deflection at a distance r from the center of symmetry due to the total surface loading $p_s(\varrho)$ is

$$w(r) = \int_0^\infty K(r,\varrho)\, p_s(\varrho)\, 2\pi\varrho\, d\varrho. \tag{3}$$

By reason of the continuity assumed to exist between the plate and the subgrade, the deflection of the plate is also given by (3). From (1) there is obtained the integro-differential equation

$$w(r) = \int_0^\infty K(r,\varrho)\,[p_0(\varrho) - N\nabla^4 w(\varrho)]\, 2\pi\varrho\, d\varrho. \tag{4}$$

GENERAL SOLUTION

Under the conditions assumed the surface loading $p_0(\varrho)$, being a bounded loading function and possessing only a finite number of finite discontinuities, may be written

$$p_0(\varrho) = \int_0^\infty P_0(\alpha)\, J_0(\alpha\varrho)\, \alpha\, d\alpha, \tag{5}$$

where

$$P_0(\alpha) = \int_0^\infty p_0(t)\, J_0(\alpha t)\, t\, dt \tag{6}$$

is the Fourier-Bessel transform[1] of $p_0(\varrho)$. One may assume the solution of (4) to be of the form

$$w(r) = \int_0^\infty W(\alpha)\, J_0(\alpha r)\, d\alpha. \tag{7}$$

Then

$$N\nabla^4 w(r) = \int_0^\infty N\alpha^4 W(\alpha)\, J_0(\alpha r)\, d\alpha$$

and these values inserted in (4) lead to

$$\int_0^\infty W(\alpha)\, J_0(\alpha r)\, d\alpha = \int_0^\infty [\alpha P_0(\alpha) - N\alpha^4 W(\alpha)]\, d\alpha \int_0^\infty 2\pi\varrho K(r,\varrho)\, J_0(\alpha\varrho)\, d\varrho, \tag{8}$$

in which an inversion of the order of integration has occurred in the right member, a process readily justifiable.

Employing the definition of $K(r,\varrho)$, the last integral of the right member of (8) may be written as $K(\alpha)\, J_0(\alpha r)$ where

$$K(\alpha) = \int_0^\infty 2\pi t\, k(t)\, J_0(\alpha t)\, dt. \tag{9}$$

Hence (8) becomes

$$\int_0^\infty [W(\alpha) - \alpha P_0(\alpha) K(\alpha) + N\alpha^4 W(\alpha) K(\alpha)] J_0(\alpha r)\, d\alpha = 0.$$

Since this is an identity in r,

$$W(\alpha) = \frac{\alpha P_0(\alpha) K(\alpha)}{1 + N\alpha^4 K(\alpha)} \tag{10}$$

From (7) the general solution for the axially symmetric case is

$$w(r) = \int_0^\infty \frac{\alpha P_0(\alpha) K(\alpha) J_0(\alpha r)\, d\alpha}{1 + N\alpha^4 K(\alpha)}. \tag{11}$$

This solution shows how the action of the plate under a given loading is influenced by altering the assumptions concerning the physical nature of the foundation, that is, if $k(\varrho)$ and consequently from (9) its transform $K(\alpha)$ is known. Otherwise for a given $k(\varrho)$ the variations due to differing types of symmetric loading may be studied.

EFFECTS OF SUBGRADE

For a rigid support $k(\varrho) = 0$ and the solution is trivial. For a floating plate the influence function is zero everywhere except at the origin where it has a singularity such that $K_1(\alpha) = 1/\varkappa$ ($\varkappa$ = const.), that is the supporting medium offers a reaction pressure proportional to the deflection. In the usual classical elasticity theory the surface deflection[2] $k(\varrho)$ of a homogeneous istotropic semi-infinite medium due to a unit load is $k_2(\varrho) = (1 - \nu_s^2)/\pi E_s \varrho$ where the subscripts refer to the moduli of the elastic supporting medium. For this isotropic case equation (9) yields $K_2(\alpha) = 2(1 - \nu_s^2)/E_s\alpha = N_s/\alpha$. For cylindrical isotropy the same type of $K_2(\alpha)$ is valid with N_s involving all the elastic constants. The general solutions (11) for these two media are

$$w_1(r) = \int_0^\infty \frac{P_0(\alpha) J_0(\alpha\varrho)\, \alpha\, d\alpha}{\varkappa + N\alpha^4}, \quad w_2(r) = N_s \int_0^\infty \frac{P_0(\alpha) J_0(\alpha r)\, d\alpha}{1 + N N_s \alpha^3}. \tag{12}$$

Special cases of these integrals have been published by several writers[3]. The present solution seems to be of wider generality in that it is possible to solve the problem explicitly for any type of supporting medium whose properties may not all be known provided the deflection of the surface due to a unit load is known.

EFFECT OF PLATE LOADING

From (6) the transform $P_0(\alpha)$ may be found for any loading $p_0(\varrho)$ which has the properties already stated. In particular for a uniform loading of intensity p_0 over a circle of radius c, one finds $P_0(\alpha) = p_0 c J_1(\alpha c)/\alpha$ and for the elastic case equation (12) becomes

$$(13) \qquad w_2(r) = N_s p_0 c \int_0^\infty \frac{J_1(\alpha c) J_0(\alpha r)\, d\alpha}{\alpha + N N_s \alpha^4}.$$

An important type of loading is that of a circular line load of constant intensity p_0 on the periphery of a circle of radius ϱ. For such a loading $P_0(\alpha) = \varrho p_0 J_0(\alpha\varrho)$ and (12) yields

$$(14) \qquad w_2(r) = N_s p_0 \varrho \int_0^\infty \frac{J_0(\alpha\varrho) J_0(\alpha r)\, d\alpha}{1 + N N_s \alpha^3}.$$

By replacing $2\pi\varrho p_0$ by P, the last integral shows the reciprocal nature of the deflection on a circle r due to a load P on a circle ϱ and vice versa. Thus one may also derive (13) by summation of ring loads as follows:

$$w_2(r) = N_s p_0 \int_0^c \int_0^\infty \frac{\varrho J_0(\alpha r) J_0(\alpha\varrho)\, d\alpha\, d\varrho}{1 + N N_s \alpha^3}$$
$$= N_s p_0 c \int_0^\infty \frac{J_1(\alpha c) J_0(\alpha r)\, d\alpha}{\alpha + N N_s \alpha^4}.$$

For a concentrated load $P_1 = \pi c^2 p_0$, one finds from the limit of (13) as c approaches zero,

$$(15) \qquad w_2(r) = \frac{N_s P_1}{2\pi} \int_0^\infty \frac{J_0(\alpha r)\, d\alpha}{1 + N N_s \alpha^3}$$

The same result may be obtained if one considers the limiting cases of shrinking the loadings of a paraboloid of revolution or hemispherical load of maximum intensity p_0. Their respective transforms are

$$P_0(\alpha) = 2 p_0 J_2(\alpha c)/\alpha^2$$

and

$$P_0(\alpha) = p_0 (c/\alpha)^{3/2} J_{3/2}(\alpha c) \sqrt{\pi/2}.$$

The deflection is then obtained from (11) or independently by addition of loads of the type given in (14).

DEFLECTION AND MOMENTS

Defining $\lambda = l\alpha$ and $l^3 = N N_s$ then (15) becomes

$$(16) \qquad w_2(r) = \frac{N_s P_1}{2\pi l} \int_0^\infty \frac{J_0(\lambda r/l)\, d\lambda}{1 + \lambda^3}.$$

At the origin this deflection is $N_s P_1 \sqrt{3}/(9l)$.

At very large distances the non-oscillatory decay of the deflection is indicated by the asymptotic character of $J_0(r) \approx \sqrt{2/\pi r}\, \cos(r - \pi/4)$ and the amplitude vanishes as $r^{-5/2}$.

The moment sum $M = -N(1+\nu)\nabla^2 w$ is

$$M = \frac{(1+\nu)P_1}{2\pi}\int_0^\infty \frac{\lambda^2 J_0(\lambda r/l)\,d\lambda}{1+\lambda^3} \tag{17}$$

thus becoming infinite at the origin and vanishing at infinity as $r^{-1/2}$.

PLANE CASE

From the axially symmetric case of (16) and (17) one may obtain the plane strain case of an infinite beam on an elastic base. One considers the effect at a point x along the beam by summing for a constant line density P from $-\infty$ to $+\infty$ on the y axis. From (16)

$$w_2(x) = \frac{N_s P_1}{2\pi l}\int_{-\infty}^{+\infty}\int_0^\infty \frac{J_0(\lambda/l\sqrt{x^2+y^2})\,d\lambda dy}{1+\lambda^3} \tag{18}$$

$$= \frac{N_s P_1}{\pi}\int_0^\infty \frac{\cos(\lambda x/l)\,d\lambda}{\lambda(1+\lambda^3)}$$

The moment in the beam may be obtained directly from (18) by differentiation. Alternatively it may be obtained from (17) for the axial case, that is by replacing $P_1/2$ by P_1 and $(\lambda/l)J_0(\lambda r/l)$ by $\cos(\lambda x/l)$, resulting in

$$M_r = \frac{P_1 l}{\pi}\int_0^\infty \frac{\lambda\cos(\lambda x/l)\,d\lambda}{1+\lambda^3}. \tag{19}$$

This integral has been treated by Biot[4] and Marguerre[3]. Integral (19) was derived from an integro-differential equation by M. E. Reissner[5].

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NOTES ON THE INTERNAL ANATOMY OF CANTHON LAEVIS DRURY

ROBERT H. COOPER[1]

From the Department of Zoology and Entomology, Iowa State College, and Ball State Teachers College, Muncie, Indiana

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Canthon laevis Drury is one of the Scarabaeidae belonging to the tumble-bug group, Coprinae. About the habits of this species much has been written, but about its morphology little seems to have been published. Hardenburg (1907) described the mouthparts of this beetle. Mohr (1929) studied the external structure of the entire insect. Willimzik (1930) described the female sex organs of a closely related species, *Scarabaeus sacer* L. Nothing appears to have been published on the internal anatomy of *Canthon laevis*.

The works of Straus-Durckheim (1828), Imms (1925), Bugnion (1933), and Weber (1933) were helpful in this study as references on the anatomy of the nervous and digestive systems of beetles in general.

The following original descriptions of the internal organs of *Canthon laevis* are based upon dissections of approximately two dozen individuals taken from Geneva, Indiana.

GENERAL DESCRIPTION OF THE NERVOUS SYSTEM

The central nervous system (fig. 1) is composed of a supra-oesophageal ganglion or brain, a suboesophageal ganglion, a thoracic ganglion, a thoracico-abdominal ganglionic center, double longitudinal cords or connectives joining these ganglia in a series, and many nerves extending from the ganglia to the different parts of the body.

The brain lies dorsally upon the oesophagus. Projecting laterally from each side of the brain is a comparatively small optic lobe. Connecting the supra-oesophageal ganglion and the suboesophageal ganglion are unusually long circumoesophageal connectives, their increased length being due to the rather large oesophagus of this beetle. The usual pairs of nerves extend to the mouth parts of the head from the suboesophageal ganglion. Parallel connectives lead caudad to the first thoracic ganglion. From this center, nerves extend to the prothoracic legs and other thoracic parts. Closely connected to the first thoracic ganglion is a larger thoracico-abdominal ganglionic center. This mass is composed of the coalesced mesothoracic, metathoracic, and abdominal ganglia and lies in the caudal portion of the prothorax and in the mesothorax. From this center, nerves extend to the mesothoracic legs, the metathoracic legs, the dorsal body region, and the abdominal region. There are no abdominal ganglia.

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GENERAL DESCRIPTION OF THE ALIMENTARY CANAL

The alimentary canal (fig. 2) consists of the very short stomodaeum, the very long mesenteron, and the proctodaeum. From the fresh specimens dissected the longest alimentary tube measured two hundred twenty millimeters and the shortest, one hundred fifty-nine millimeters. The former was 11.15 times the total body length.

The stomodaeum consists of the pharynx and the oesophagus. The length of the stomodaeum is approximately 2 per cent of the total length of the canal. The oesophagus leads directly posteriorly to the mesenteron, there being no crop or proventriculus. An internal view shows folds of the stomodaeum treminating as the oesophageal valve.

The mesenteron or ventriculus consists of the anterior mesenteron, regenerative crypts or villiform gastric caeca, and the posterior mesenteron. The entire mid-gut makes up about 90 per cent of the total canal. The anterior mesenteron lies in the thoracic region and averages 3.5 per cent of the total ventriculus. The posterior mesenteron lies in the abdominal cavity and is slightly narrower than the anterior mesenteron. Its width is approximately one millimeter. It is coiled many times, giving an appearance similar to that of a watch-spring. Among these coils and attached outside the digestive canal are numerous fat bodies (fig. 4) and tracheae.

Numerous regenerative crypts or gastric caeca of uniform size project out from the surface along the entire length of the mesenteron. A transverse section (fig. 3) shows the crypts are constricted near their connections with the mesenteron wall. The inner cellular structure appears to be continuous with that of the epithelium of the mesenteron.

A microscopic study of a series of cross sections of the anterior mesenteron (fig. 3) shows an epithelium, a basement membrane, a circular muscle layer, a longitudinal muscle layer, and an outside peritoneal layer. An external view of a longitudinal section (fig. 4) of the posterior mesenteron shows the evaginations which form several regenerative crypts and the tracheal attachment of a fat body.

The proctodaeum averages 8 per cent of the entire canal. It consists of the pyloric valve, the colon, and the rectum. It increases in breadth from the valvular region to near the anus where it reaches a width of approximately three millimeters. There are four Malpighian tubes.

There is a translucent, smooth, and somewhat constricted part of the proctodaeum extending about three millimeters caudad from the base of the Malpighian tubules. Internally, there are folds of the proctodaeum which give this translucent region the appearance of a valve. The author has termed this the region of the pyloric valve. The remainder of the intestinal wall is marked only by the presence of numerous large external ridges. Ventral to the rectum are the ducts of the sex organs.

FAT BODIES

As systems are dissected from specimens of *Canthon laevis* masses of fat bodies, arranged like link sausages (fig. 5) obscure the view and bother continually. Some of these give the deceptive appearance of numerous long caeca protruding from the food tube (fig. 4). Many fat bodies are attached also to the walls of the abdominal cavity.

REPRODUCTIVE SYSTEM

The female reproductive system consists of an ovary, oviduct, vagina, and a spermatheca (fig. 6). The single ovary (left) consists of a single ovariole having a terminal filament followed posteriorly by the germarium and vitellarium. A very short oviduct leads to the vagina. The tubular spermatheca is attached at the left side and extends from left to right dorsally over the vagina. The right ovary and its oviduct are absent.

The male reproductive system (fig. 7) consists of paired testes, accessory glands, vasa deferentia, an ejaculatory duct, and the aedeagus. This system is jammed together under the last two or three abdominal tergites. Lying anterior to the other parts of the system are the paired accessory glands. Ventrad of all other parts of the reproductive system are the testes. Each testis consists of six rather spherical follicles. These are bound together into a mass by an orange tinted tissue. A vas deferens leads from each testis and joins the ejaculatory duct at a point very near that to which the previously described paired accessory glands are attached. Extending forward from the anterior end of the curved ejaculatory duct is a single tubular accessory gland. The ejaculatory duct terminates posteriorly in the male intromittent organ or aedeagus.

TRACHEAL SYSTEM

The respiratory system consists of eight spiracles, tracheae, air sacs, and tracheoles. A dorsal view (fig. 8) shows that the abdominal tracheae have no expansions or air sacs. In the posterior margin of the thoracic region there is a mass of air sacs which hangs slightly into the anterior of the abdominal cavity. Tracheae from these sacs and from the mesothoracic spriracles lead anteriorly between the thickly muscled meta- and mesothoracic body walls to become the dorsal and ventral prothoracic air sacs. The prothoracic sacs taper cephalad into the head, where branches of the tracheae reach to all parts.

A lateral view of the abdominal tracheae in the region of the spiracles shows the large, lateral tracheal trunk from which branches extend throughout the abdomen. The posterior end of the trunk is especially prominent because of its numerous and bunched branching.

A caudal view (fig. 9) of the group of air sacs in the metathoracic region shows that there are seven pairs of sacs. The largest of these originates directly from the large metathoracic spiracles. The three pairs of small sacs originate as branches from the larger sacs.

SUMMARY

1. The abdominal ganglia of the nervous system are coalesced with the meso- and metathoracic ganglia to form a thoracico-abdominal ganglionic center.

2. The stomodaeum has neither crop nor proventriculus. The mesenteron is exceedingly long in comparison to the total length of the insect body and has projecting regenerative crypts or gastric caeca over its entire length.

3. All of the body cavities are lined with sausage-shaped fat bodies formed around tracheal branches.

4. The female reproductive system has only the left ovary developed with but a single ovariole. The oviduct is very short.

5. The male reproductive system is crowded into a small space far to the rear of the abdominal cavity. Beneath the paired accessory glands which make up the greater part of the bulk of this system are the small rounded testes. An unpaired accessory gland lies at the proximal end of the ejaculatory duct.

6. The respiratory system shows no air sacs arising from the abdominal tracheae, but seven pairs arise from the metathoracic spiracles and form a group caudad to the heavily muscled thoracic region. Air sacs from the mesothoracic spiracles extend forward into the head.

PLATE I

Explanation of figures

Fig. 1. A dorsal view of the central nervous system (1.5x). supra. g., super-oesophageal ganglion; o. l., optic lobe; o. c., circumoesophageal connective; s. g., suboesophageal ganglion; c., connective; l., nerve to leg; th., thoracic ganglion; th. g. c., thoracico-abdominal ganglionic center; d., nerves to dorsal region; a., abdominal nerves.

Fig. 2. A dorsal view of the alimentary canal (1.5x). ph., pharynx; oe., oesophagus; o. v., oesophageal valve; a. m., anterior mesenteron; v. g. c., regenerative crypt or gastric caecum; p. m. posterior mesenteron; m. Malpighian tubes; p. v., pyloric valve; co., colon; r., rectum; an., anus; A., stomodaeum; B., mesenteron; C., proctodaeum.

Fig. 3. A transverse section through the anterior mesentron (12x). v. g. c., regenerative crpyt or gastric caecum; epi., epithelium; l. m., longitudinal muscle; c. m., circular muscle; b. m., basement membrane.

Fig. 4. A longitudinal view of a portion of the posterior mesenteron (9x). f. b., fat body; v. g.c., regenerative crypt or gastric caecum; tch., trachea; c. m., circular muscle; l. m., longitudinal muscle.

Fig. 5. A diagram of fat bodies on the tracheae (9.4x). tra., trachea; tch., tracheal branches; f. b., fat body.

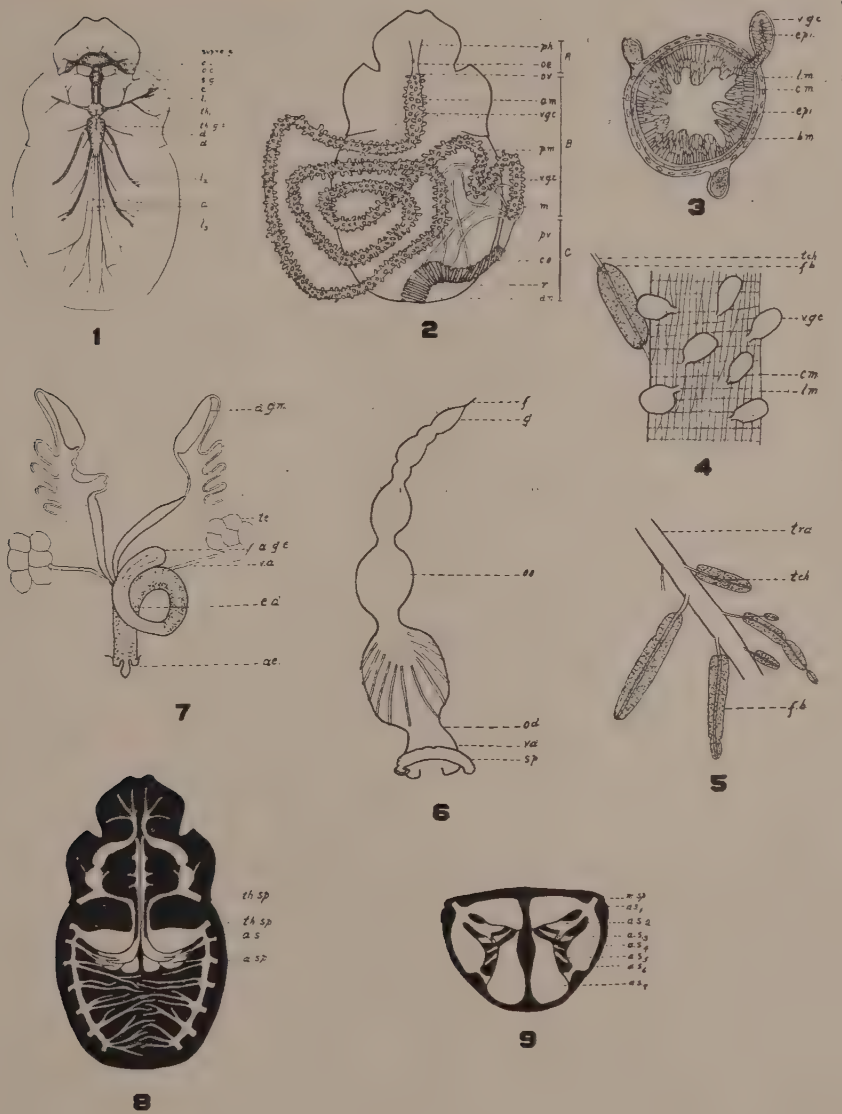
Fig. 6. A dorsal view of the female reproductive system (4.1x). f. filament; g., germarium; oo., oöcyte; od., oviduct; va., vagina; sp., spermatheca.

Fig. 7. A dorsal view of the male reproductive system with the parts stretched out (3x). a. g. m., anterior paired accessory glands; te., testis; a. g. e., posterior single accessory gland; v. d., vas deferens; e. d., ejaculatory duct; ae., aedeagus.

Fig. 8. A dorsal view of the respiratory system with obstructing organs removed and the tracheae and air sacs shown in white (1.5x). th. sp., thoracic spiracle; a. s., air sac; a. sp., abdominal spiracle.

Fig. 9. A caudal view of the metathoracic air sacs (1.5x). m. sp., metathoracic spiracle; a. s., air sac.

PLATE I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EFFECT OF FUMIGATION OF WHEAT ON AMYLASE CONTENT

VERA DAWSON MARTIN[1]

From the Departments of Chemistry, Zoology and Entomology, Iowa State College

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As pointed out by Mangels (1) the diastatic activity of wheat is very important in the baking quality of the flour that is milled from it. Since it has become a common practice to fumigate wheat in storage against insects and fungi, the question arises whether the fumigation produces a deleterious effect on the diastatic activity of the wheat. Fumigation of wheat with sulfur dioxide (2), for instance, is known to lower markedly the baking quality of the flour milled from it. This investigation was undertaken to prove whether the effect of sulfur dioxide and that of other fumigants are due to injury to the diastase of wheat.

Wheat samples were fumigated with 15 different fumigants under conditions approximating those used in large bins, and the diastatic activity determined on water extracts of the ground grain. The fumigants tested include some that are in common use and some which are still in the experimental stage.

The grain used was No. 1 hard winter wheat, variety Iobred x Minhardi, from the 1936 crop of the Agronomy Department of Iowa State College. In one series of experiments soft wheat was used in order to establish whether there was a difference in resistance to penetration of the fumigants. The fumigations with volatile liquids were carried out in 4-liter Erlenmeyer flasks using 1000 grams of grain. The technique was that of Naylor and Dawson (3), in which the calculated amounts of volatile liquids were measured into test tubes, dropped in on the grain, and the flasks stoppered and sealed with collodion. Fumigants applied in this manner were carbon disulfide, ethylene oxide, chloropicrin, ethylene dichloride-carbon tetrachloride mixtures, ethylene dichloride, carbon tetrachloride, methyl bromide, methyl formate and methyl thiocyanate.

The vapors for fumigations with hydrocyanic acid, sulfur dioxide and hydrogen sulfide were generated in the flasks by chemical reactions. A 50-cc. beaker was suspended by wires from the stopper of a 4-liter suction flask carrying a dropping funnel. For the hydrocyanic acid fumigation, solid potassium cyanide and a few drops of water were placed in the beaker and a slight vacuum drawn on the flask. Sulfuric acid was allowed to drop onto potassium cyanide and after all the acid had been added the stopcock was left open a few seconds to restore the atmospheric pressure within the flask. For generating sulfur dioxide, sodium sulfite was placed in the small beaker, and sulfuric acid added as for the hydrocyanic acid. The same technique was used for generating hydrogen sulfide from ferrous sulfide.

For the fumigation with naphthalene, an excess of the solid was placed in the beaker in the suction flask as for generating vapors by a

[1] The author wishes to thank Professor R. M. Hixon for suggesting the problem and Professor C. H. Richardson for directing the work.

chemical reaction. The evacuated flask was allowed to stand several hours at 30° C. to allow sublimation to take place, and then air was let in slowly. The air in the flask was saturated with naphthalene and according to Lehman (4) would contain 0.000852 g. per liter.

An atmosphere of carbon dioxide was obtained over the grain in a suction flask by evacuating with a water pump, then running carbon dioxide from a tank through sulfuric acid and into the flask. This was repeated three times. The flask was then left overnight with carbon dioxide gas going through at the rate of four liters per hour. The exit gas was led into a nitrometer containing 50 per cent potassium hydroxide. Six cubic centimeters of gas insoluble in potassium hydroxide was collected in one hour. From this the gas in the flask was calculated to be 99.8 per cent carbon dioxide.

Fumigation with dry heat was accomplished by placing 500 g. of grain in a large dish in an oven at 150° F. for three hours (2).

The flasks of wheat containing the fumigants were placed in an incubator at 25° C. for 24 hours, except in the case of the naphthalene and dry heat treatments. The flasks were opened after 24 hours and the gases allowed to escape another 24 hours. The fumigated wheat samples were prepared for amylase determinations by grinding completely through the No. 2 screen of the Wiley mill.

The amylase content of the wheat samples was determined by a method similar to that of Gore (5), since the ungerminated grain contains only the saccharifying amylase (6). Twenty-gram samples of the ground wheat were weighed into beakers and 50 cc. of ice cold distilled water added, and the mixtures allowed to stand overnight in the icebox. The extracts were filtered off with suction and diluted 1-5 with ice cold distilled water. Five cubic centimeters of each extract was added to 100 cc. of a 2 per cent suspension of soluble starch which was buffered to pH 5 with phosphate buffers. These mixtures were placed in a thermostat at 40° C. After 30 minutes 5.0 cc. portions were pipetted from each digestion into 25 cc. of alkaline ferricyanide reagent in 250 cc. E. flasks. These flasks were placed in a boiling water bath 15 minutes, cooled and 25 cc. of acetic acid reagent added. Five cubic centimeters of 50 per cent potassium iodide solution was added and the iodine set free titrated with standard 0.1N sodium thiosulfate solution[1]. The starch remaining in the sample serves as an indicator.

The titration results were coverted to milligrams of maltose by reading directly from a reference curve of cubic centimeters of 0.1N sodium thiosulfate against milligrams of maltose. This curve was prepared by titration of known samples of C. P. maltose hydrate whose purity had been checked by the Munsen-Walker method.

When soft wheat was fumigated with five times the recommended amount of carbon disulfide the amylase value was 98.6, with the control value on the untreated soft wheat as 100. Fumigation with five times

[1] Reagents—

Alkaline ferricyanide reagent—0.1N solution in 5 per cent sodium carbonate.
Acetic acid reagent—200 cc. of glacial acetic acid, 80 g. of $ZnSO_4 \cdot 7H_2O$ and 70 g. of KCl and water to make one liter.
Potassium iodide solution—50 per cent solution containing one drop concentrated NaOH per 100 cc.

the usual amount of ethylene oxide gave a value of 98.5 and with hydrocyanic acid 97.3. There was no significant deleterious effect on the amylase present in the soft wheat when the grain was treated with these fumigants.

Table 1 shows the results obtained when hard winter wheat was treated with the frequently recommended amounts of fumigants (amounts and references to the literature are shown in the table) and also a second series when the amount of each fumigant was increased five times. The extractions and tests were run in triplicate on each sample of fumigated grain. Controls were run on the untreated grain every time new samples of fumigated grain were tested. Setting the average for the values on controls at 100 and dividing by the average for the determinations on the samples by that for the controls run at the same time, the whole set of data becomes comparable.

The data given in the table show that in no case is the deviation of the samples of hard wheat from the controls of untreated hard wheat greater than 5 per cent, even when five times the recommended amounts of fumigants were used. The precision of the method of determining diastase activity by water extraction is not less than five per cent. The deviations shown in the table are due to differences in the amounts of enzyme extracted, because the determination of reducing sugars is reproducible to

TABLE 1. *Amylase content of fumigated wheat*

Fumigant	Recommended Amount with Literature Reference	Series I*		Series II**	
		Maltose Formed	Control as 100	Maltose Formed	Control as 100
		mg.		mg.	
Control	not fumigated	41.5	100	42.8	100
Carbon disulfide	20 lbs. per. 1,000 cu. ft. (7, 8, 10)	40.6	98.0	42.2	98.5
Carbon dioxide	99.8 per cent atmosphere	42.0	101.2		
Ethylene oxide	2 lbs. per 1,000 cu. ft. (7, 9, 10)	40.0	96.5	42.2	98.5
Hydrocyanic acid	2:3:4 formula (7)	41.0	98.8	41.3	96.5
Control		55.3	100	49.0	100
Ethylene dichloride and carbon tetrachloride	20 lbs. of 3-1 mixture per 1,000 cu. ft. (7, 8, 10)	57.5	104	49.3	100.3
Ethylene dichloride	Same amount as above	58.1	105	48.6	99.9
Carbon tetrachloride	Same as in mixture	57.5	104	48.6	100.1
Sulfur dioxide	pot method (7)	58.5	105.5	49.6	100.3
Chloropicrin	1 lb. per 1,000 cu. ft. (7, 11)	55.4	96.5	49.4	100.5
Controls		49.0	100	49.0	100
Methyl bromide	Twice the HCN dose	49.2	100.2	49.7	100.2
Hydrogen sulfide	25 per cent atmosphere (2)	49.2	100.2		
Naphthalene	Saturated atmos. at 30°C (4)	49.3	100.3		
Dry heat	150°F. for three hours (2)	48.9	99.8		
Controls		56.5	100	56.5	100
Methyl formate	25 mg. per L. (12)	56.0	99.5	56.7	100.2
Controls		49.5	100	49.5	100
Methyl thiocyanate	10 mg. per L. (13)	50.1	101.1	50.2	101.5

Note:
* Fumigated with recommended amounts.
** 5 times recommended amounts of fumigants.

0.5 per cent. The wide variation in the controls run on different days is the result of differences in the moisture content of the ground grain owing to differences in humidity.

The results of these experiments on the effect of fumigation on the saccharifying amylase present in wheat can be summarized as follows:

1. When the grain was treated with the frequently recommended amounts of fumigants the amylase was not injured.

2. The amylase present was not injured by increasing the amounts of fumigants five times.

3. That the absence of harmful effects of fumigation is not due to a varietal resistance is shown by the fact that there was no inhibition of enzyme activity in soft wheat after fumigation.

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ZEROS OF A CLASS OF POLYNOMIALS ASSOCIATED WITH BATEMAN'S k-FUNCTION

J. M. Bates

From the Department of Mathematics, Iowa State College

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Consider the class of polynomials defined by the relation

$$e^{\frac{2Sx}{1+S}} = \sum_{n=0}^{\infty} U_n(x) S^n,$$

where $U_n(x)$ is the nth polynomial. These polynomials are closely related to Bateman's k-function[1], which is of importance in the theory of turbulence, and is a special type of the Wittaker function $W_{k,m}(z)$ corresponding to the case $m = 1/2$.

The differential equation and the corresponding difference equation which are satisfied by these polynomials may be obtained from the generating function by the usual methods. Thus, we have

$$xU_n''(x) - 2xU_n'(x) + 2nU_n(x) = 0,$$

$$2(x-n)U_n(x) = (n+1)U_{n+1}(x) + (n-1)U_{n-1}(x).$$

The term containing the first derivative of $U_n(x)$ in the differential equation can be eliminated by making the substitution

$$U_n(x) = e^x V_n(x),$$

where $V_n(x)$ is the even ordered Bateman k-function. The differential equation obtained, which is satisfied by $V_n(x)$, is

$$(1) \qquad xV_n''(x) + (2n - x)V_n(x) = 0.$$

It is obvious that $U_n(x)$ and $V_n(x)$ vanish for the same values of the variable. Two asymptotic expansions will be determined for the zeros of $V_n(x)$ one of which is especially adapted for the computation of the small zeros and the other for the computation of the large zeros.

O. Bottema[2] has presented a method for obtaining the limits for the zeros of a function $Q_n(x)$ which satisfies a difference equation of the type

$$Q_n(x) - (x + b_n)Q_{n-1}(x) + C^2_{n-1}Q_{n-1}(x) = 0.$$

If the results of his work are used the limits for the zeros of $U_n(x)$ can be expressed as

$$(2) \qquad (U_n) = -\frac{1}{2}(b_n + b_{n+1}) \pm \sqrt{\frac{1}{4}(b_n - b_{n+1})^2 + \frac{4C_n^2}{(1-k_n)(1+k_{n+1})}}$$

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where $b_n = (1-n)$, $C_n = \frac{1}{2}\sqrt{n(n-1)}$, and $|k_n| < 1$. The positive sign is used before the radical to obtain an upper limit and the negative sign is used to obtain a lower limit. If the values for b_n and c_n, along with the condition $k_n = 0$, are substituted in (2) the interval for the zeros of $U_n(x)$ is found to be $0 \leqq x < 2n - 1$.

The small zeros of $U_n(x)$. To obtain the small zeros of $V_n(x)$ we first derive an expansion for $V_n(x)$ valid in the neighborhood of the origin. In order to cut down the interval in which the zeros lie, the transformation

$$t = 2nx$$

may be made in the differential equation

$$xV_n''(x) + (2n - x)V_n(x) = 0. \tag{3}$$

The resulting differential equation is

$$tV_n''(t) + (1 - \lambda t)V_n(t) = 0, \tag{4}$$

where $\lambda = 1/(2n)^2$. From equation (4) two independent solutions for $V_n(t)$ can be found by the method of Frobenius. In these solutions the coefficients of the powers of t are polynomials in λ and the degree of these polynomials in λ increases as the power of t increases. Therefore, a solution can be expressed as a power series in λ. Hence,

$$V_n(t) = \sum_{i=0}^{\infty} \omega_i \lambda^i, \tag{5}$$

where ω_i is a function of t. The coefficients of the powers of λ must vanish identically if (5) is to be a particular solution of equation (4). The conditions that the coefficients vanish are

$$\begin{aligned} t\omega_0'' + \omega_0 &= 0, \\ t\omega_1'' + \omega_1 &= t\omega_0, \\ \cdot\ \cdot\ \cdot\ &\cdot\ \cdot\ \cdot\ \cdot\ , \\ t\omega_i'' + \omega_i &= t\omega_{i-1}, \\ \cdot\ \cdot\ \cdot\ &\cdot\ \cdot\ \cdot\ \cdot \end{aligned} \tag{6}$$

A particular solution for the first differential equation of this system is readily seen to be

$$\omega_0 = c_1\sqrt{t}\,J_1(2\sqrt{t}\,),$$

where J_1 is a Bessel function. Therefore, we may assume that

$$\omega_i = p_i\omega_0 + q_i\omega_0',$$

where p_i and q_i are polynomials in t. Equations (6) may be solved for ω_i which satisfies the boundary conditions

$$q_i = 0,\ p_i + q_i' = 0, \text{ when } t = 0.$$

The zeros of $V_n(t)$ may be assumed to be of the form $\alpha + h$ where α is a zero of ω_0, and

$$h = \sum_{i=1}^{\infty} C_i \lambda^i,$$

in which C_i is a polynomial in α. If $V_n(\alpha + h)$ is expanded by Taylor's formula we obtain

$$V_n(\alpha + h) = V_n(\alpha) + V_n'(\alpha)h + V_n''(\alpha)\frac{h^2}{2!} + \cdots\cdots = 0. \tag{7}$$

The conditions for the vanishing of the coefficients of powers of λ in equation (7), provide us with a system of equations from which C_i may be found. Thus the expansion for the small zeros of $U_n(t)$ takes the form

$$\text{Zero} = \alpha + \frac{\alpha^2}{3}\lambda + \left[\frac{11\alpha^3}{45} - \frac{2\alpha^2}{15}\right]\lambda^2 + \left[\frac{73\alpha^4}{315} - \frac{12\alpha^3}{35} + \frac{16\alpha^2}{63}\right]\lambda^3 + \left[\frac{3548\alpha^5}{14175} - \frac{664\alpha^4}{945} + \frac{5925\alpha^3}{4725} - \frac{16\alpha^2}{15}\right]\lambda^4 + \cdots\cdots \tag{8}$$

Values of α may be obtained from Watson's tables[3] and the first five are

$$\begin{aligned} \alpha_1 &= 3.670492, \\ \alpha_2 &= 12.304614, \\ \alpha_3 &= 25.874863, \\ \alpha_4 &= 44.380191, \\ \alpha_5 &= 67.820413, \\ &\cdots\cdots \end{aligned}$$

The large zeros of $U_n(x)$. If the transformation

$$y = \frac{2n - x}{(2n)^{1/3}}$$

is made in the differential equation (1) the resulting differential equation is

$$(1 - \mu y)V_n''(y) + yV_n(y) = 0, \tag{9}$$

where $\mu = 1/(2n)^{2/3}$. From this point the procedure is similar to that used for the expansion of the small zeros and we obtain the following expansion for the large zeros of $U_n(x)$:

$$\text{Zero} = \beta - \frac{\beta^2}{5}\mu + \left[\frac{6}{35} - \frac{3\beta^3}{175}\right]\mu^2 + \left[\frac{8\beta}{525} - \frac{46\beta^4}{2625}\right]\mu^3 - \left[\frac{5326\beta^2}{606385} - \frac{17356\beta^5}{3031875}\right]\mu^4 + \cdots\cdots, \tag{10}$$

[3] Watson, G. N. A Treatise on the Theory of Bessel Functions. p. 748. Cambridge University Press. 1922.

where β is a zero of $J_{1/3}(2/3\, x^{3/2}) + J_{-1/3}\,(2/3\, x^{3/2})$. The first five values for β as obtained from Watson's tables are

$$\begin{aligned}
\beta_1 &= 2.338107,\\
\beta_2 &= 4.137258,\\
\beta_3 &= 5.520555,\\
\beta_4 &= 6.786701,\\
\beta_5 &= 7.944136,\\
&\cdots\cdots
\end{aligned}$$

The first five and the last five zeros, for $n = 40$, as calculated from (8) and (10), respectively, are

0.045889,	. . . . ,
0.153906,	48.843598,
0.323873,	52.981174,
0.556041,	57.670743,
0.850773,	62.971927,
. . . . ,	70.170069.

HYGROSCOPICITY AS A FACTOR IN THE THERMAL CONDUCTIVITY OF LOOSE-FILL INSULATORS[1]

H. E. Ruff

From the Department of Physics, Iowa State College

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In recent years the condensation of moisture within the walls of structures employing loose-fill insulation has become a matter of grave concern. The problem had its inception with the comparatively recent improvement in building practices and there are three things which combine to aggravate the situation: tighter construction, thermal insulation, and air conditioning.

While experimenting with means for the prevention of moisture accumulation on interior building surfaces Close (3) found that water vapor will penetrate any loose-fill insulating material and condense at the dew point. McPherson (6,7) observed that such moisture accumulation in loose-fill thermal insulation will continue indefinitely if there is any appreciable infiltration of air through the walls and thus the insulation material will eventually reach a state of complete saturation. Teesdale (11) demonstrated that differences in vapor pressures on the inside and outside of walls will result from weather conditions frequently encountered in the winter season thus causing a vapor movement through the walls with attendant condensation. He further showed that these phenomena are independent of air movement to the degree that no general circulation of air is necessary to carry the vapor into the wall.

Investigations by Batsch (1), Madgwick (5), Rowley (8, 9), Berestneff (2), Finck (4), and Schmidt (10) have, on the whole, borne out the accuracy of the conclusions stated in the foregoing paragraph.

At the present time exhaustive tests of "vapor barriers" are being made by the Engineering Department of the University of Minnesota and by the United States Forest Products Laboratory at Madison, Wisconsin. Since the methods thus far devised are only partially successful in making the walls impervious to moisture vapor there appears to be an urgent need for more detailed data regarding the effect of moisture content on the thermal conductivities of the various loose-fill insulators. This need has been pointed out by Berestneff (2), by Finck (4), and by Close (3).

It is generally known that the thermal conductivity of an insulator increases with increased moisture content but the magnitude of this effect over wide ranges has never been investigated. Only two meager reports on the subject are to be found in the literature. Testing over-all heat transmission of wall structures by means of a hot box apparatus, Rowley (8) and his associates found that various types of walls filled with porous gypsum material had their transmission coefficients reduced considerably as a result of drying over a period of four or six weeks. Finck (4) investigated the effect of moisture on bagasse, cornstalk pulp, and wood pulp.

[1] A thesis submitted to the Graduate Faculty of Iowa State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Doctoral Thesis No. 466. Submitted June, 1938.

He found their conductivities to be increased less than ten per cent by several days conditioning in an atmosphere which was in equilibrium with a saturated solution of $Ca(NO_3)_2$, giving a relative humidity of approximately 53 per cent at room temperature.

In the current project moisture contents up to a maximum of forty-one per cent by weight were given to various types of loose-fill insulators and the effect on thermal conductivity noted. In successive tests for any given type the moisture content was increased to such a point as to render the material completely ineffective as thermal insulation.

DESCRIPTION OF APPARATUS

The guarded hot plate apparatus used is a modification of the standard form of apparatus described by van Dusen (12) and used by the Bureau of Standards. The changes in design from the standard form of hot plate apparatus are three-fold: (a) Method of suspending the heating units; (b) Employment of a compensating unit; (c) Structure of the cold plate tank.

Figure 1 shows a schematic diagram of the assembled apparatus. A main heating unit, E, and a compensating unit, C, are fastened with copper machine screws to bakelite lugs, A, which serve to suspend the assembled units between two threaded rods, G, attached to the supporting cabinet, K. The assembled heating units are secured by thin nuts placed on both sides of the bakelite lugs. This method of rigid suspension of the apparatus permits of making conductivity tests with the sample either in the vertical or the horizontal position, gives ready access to the sample frame, and makes the use of edge insulation more effective.

Figure 2 gives the details of construction of compensatory and main heating units and indicates the manner of assembly of their various parts.

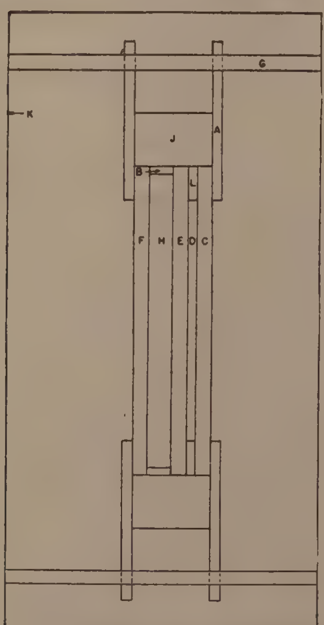

Fig. 1. Assembled apparatus

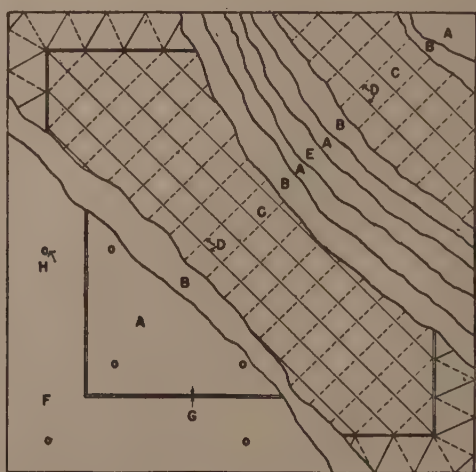

Fig. 2. Details of construction of heating units

The compensating unit consists of two square, copper plates, A, between which there is a heating element made of nichrome ribbon, D, wound on a square mica plate, C. The heating element is electrically insulated from the metal plates by thin micanite sheets, B, of slightly larger size. The main heating unit is of similar construction but has the following differences: (a) A 2 mm. saw-cut, G, isolates an outer guard ring, F, from the main heating plate thus minimizing the conduction of heat from the periphery of the hot plate; (b) The edge of the main heating element is surrounded by an auxiliary heating coil in order to compensate for heat losses from the edge of the hot plate. The employment of a compensating heating unit obviates the necessity of constructing two identical samples of thermal insulation.

The cold plate serves as one side of a shallow, square, copper tank fastened to the supporting cabinet. Staggered baffle plates evenly spaced within the tank insure an equalized flow of water to all parts of the tank and consequently a uniform temperature distribution over the entire surface of the cold plate.

The loose-fill insulation comprising the sample to be tested in placed in a hollow square made of micarta and having a depth of 2.6 cm. Thin, semi-rigid sheets of micarta fastened to the top and bottom of the hollow square by means of friction tape facilitate the handling of loose-fill materials. After the filled sample frame has been placed between the hot plate and the cold plate these thin micarta sheets are removed, thus allowing the loose-fill insulation to come into direct contact with the metal plates.

Edge insulation consisting of a framework of corkboard is placed over the assembled heating units, sample frame, and cold tank. The inner side of the edge insulation is upholstered with asbestos cloth to insure close contact with the edges of the plates and prevent the circulation of air between the various units.

Temperatures are measured by means of copper-constantan thermocouples, the electromotive forces being ascertained by means of a potentiometer arrangement. The thermo-junctions are made by soldering Number 24, B. and S. gauge, constantan wire to the copper plates. An insulated constantan wire attached to the center of the hot plate passes between the copper plate and the micanite sheet to the edge of the main heating unit and thence to a binding post on the supporting cabinet. A similar constantan wire attached to the center of the cold plate and passing through the cold tank to another binding post completes the couple. A fine copper wire makes electrical connection between the hot and cold plates.

The null method is used in securing thermal equilibrium between the main heating unit and the compensatory unit, and between the guard ring and hot plate. For this purpose banks of thermocouples in parallel are used. The manner of construction of these thermocouples is identical with that of the thermocouple between the hot and cold plate. The junctions are symmetrically located on the plates, those on the hot plate and guard ring being in close proximity to the saw-cut, and those on the main unit and compensatory unit being located on the innermost plates of these units. All circuits terminate at binding posts on the supporting cabinet. The thermocouple circuits are shown in figure 3.

As indicated in figure 2, the edge of the main heating element extends well beyond the edge of the hot plate. Only the actual length of

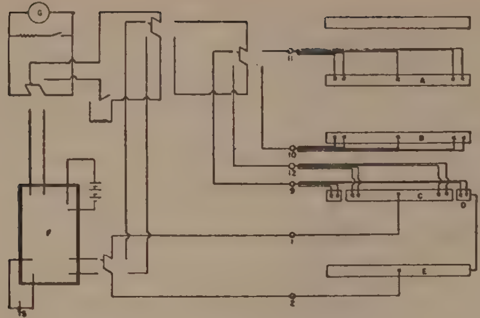

Fig. 3. Thermocouple circuits

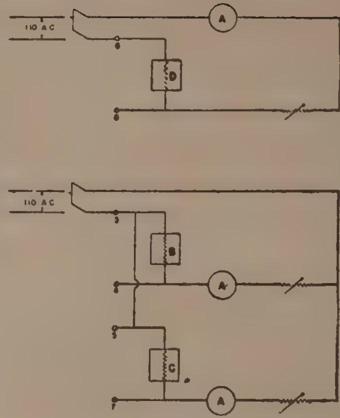

Fig. 4. Electrical connections

nichrome ribbon directly opposite the hot plate is used to determine the rate of energy supply to the hot plate. Energy is supplied to the heating elements by means of 110 volt A.C. Figure 4 gives a diagram of the electrical connections.

THEORY

The efficiency of loose-fill thermal insulation is due largely to the small dead air cells either within or between the fibers or particles of the material. Next to a vacuum, the dead air cells reduce conduction to a minimum but the presence of the loose-fill insulation also greatly deters loss of heat by convection and radiation. The transfer of heat by conduction is augmented by the surface films of water which reduce the contact resistance between the fibers or particles.

Most loose-fill thermal insulators are made of materials which are only slightly hygroscopic. However, the structure of the fabricated product is such as to endow it with a great capacity for taking up water. As a result of the physical phenomena of surface tension and capillarity some of the widely used thermal insulators are capable of holding several times their own weight of water. In this discussion all statements pertaining to hygroscopity refer to the processed insulation and not to the basic material out of which it is made.

In the apparatus described above the cold water carries the heat away as fast as it reaches the cold plate and, since the hot plate furnishes a constant supply of heat, a steady state is attained within the insulating material. Under these conditions, the time rate of heat flow, H, through the sample directly proportional to the area, A, of the hot plate and to the temperature gradient, dT/dx; hence one has

$$H = KA \frac{dT}{dx} \qquad (1)$$

where K is a proportionality constant depending on the particular type of sample under consideration. In this apparatus the temperature gradient is constant, that is, the relation between temperature and distance is

linear, and since K is approximately constant throughout short ranges of temperature, equation (1) may be written

$$H = KA\frac{T_2 - T_1}{d} \qquad (2)$$

where T_2 is the temperature of the hot plate, T_1 is the temperature of the cold plate, and d is the distance in centimeters between the plates.

EXPERIMENTAL PROCEDURE

A given sample of loose-fill insulation was placed in a vacuum desiccator for twenty-four hours and then weighed. Immediately after weighing it was given approximately the desired moisture content by means of an atomizer spray, the sample being thoroughly agitated throughout the spraying period. The material was next allowed to stand in a sealed Erlenmeyer flask of large capacity for a period of forty-eight hours or more thus permitting thorough diffusion of the moisture. The conditioned loose-fill insulation was put in the sample frame and placed in the hot plate apparatus. After determination of its thermal conductivity the sample was removed immediately from the apparatus and its moisture content ascertained by weighing on an A. Rüprecht balance.

Before a sample was placed in the thermal conductivity apparatus, the hot plate was heated to about the desired temperature and the rheostats, which were connected in series with the heating elements (see figure 4), were adjusted in such a manner as to secure an approximate state of thermal equilibrium between the main unit and the compensatory unit, and between the hot plate and the guard ring. This preliminary measure reduced to five hours the time required for the flow of heat through the sample to reach a steady state and thus minimized the loss of moisture from the sample due to evaporation.

A Weston A.C. ammeter which had been checked in the Physics Department Standards Laboratory was used to measure the power supplied to the main heating coil. A potentiometer arrangement was employed to measure the electromotive force of the thermocouple between hot plate and cold plate. The relation between thermal electromotive force and temperature was taken from a calibration curve for copper-constantan.

SOURCES OF ERROR

The probable sources of error in these investigations will be discussed as follows:

(a) Errors in the measurement of power supplied to that portion of the main heating element directly opposite the hot plate;
(b) Errors in the measurement of temperature;
(c) Errors in the measurement of thickness of sample;
(d) Errors inherent in the type of insulation tested;
(e) Thermal leakage.

The measurement of the power supplied involved the measurement of the current flowing in the main heating element and the determination of the length of resistance ribbon directly opposite the hot plate. The

error introduced in determining the current was probably small as the accuracy of the ammeter used was well within the limit of accuracy of the apparatus considered as a whole. The precision of measurement of the length of resistance wire was as great as could be secured through the careful use of a steel metric rule as the measuring device. The length was calculated from measurements made before the element was wound and checked by a measurement made before winding. Computations of the total resistance made on the bases of these two measurements differed by less than 0.1 ohm.

Errors in the measurement of temperature were small since the precision of the White potentiometer was all that could be desired. The accuracy of the thermocouples built into the apparatus was checked in the following manner. These thermocouples were used in conjunction with the potentiometer circuit to obtain data for cooling curves of the apparatus. The built-in thermocouples were then replaced by standard wire thermocouples and another series of cooling curves obtained. In no case did corresponding cooling curves of these two series differ by more than one per cent.

The thickness of a sample was taken as the depth of the micarta sample frame and this dimension was quite accurately determined through the use of a vernier caliper. Since the principal virtue of loose-fill insulation lies in the entrapped dead air cells one is justified in assuming that the interstices between particles contiguous to the hot plates are themselves constituent parts of the thermal insulation sample under consideration.

Additional errors may have resulted from the inherent nature of the type of insulation tested. It is entirely possible that the mathematical equations for heat conduction do not hold so rigidly for insulators of the loose-fill type as for solids in which the structure, or grain, is exceedingly fine compared to the total thickness of the sample. Tests on samples composed of different sizes of particles did reveal a definite variation in thermal conductivity but it is not known to what extent this variation was influenced by size of particles.

An important source of error was the transfer of heat by evaporation at the hot plate and condensation at the cold plate while testing samples of relatively large moisture content. To lessen this error all tests were made with the hot plate at a temperature about ten degrees above room temperature and the cold plate temperature about ten degrees below that of the room. Preliminary tests proved this to be the optimum temperature range consistent with minimum time requirements for reaching a steady state in the sample.

It is quite likely that the greatest source of error was that caused by thermal leakage, although every available means was employed to guard against this defect. There were no facilities at hand for keeping the temperature of the room constant and the edge insulation used proved inadequate in the attempt to secure and maintain thermal equilibrium. For the duration of any test temperature differences in the null circuits varied from two degrees downward to zero degrees.

Although use was made of a guard ring at the edge of the hot plate and a compensatory heating unit was employed to insure the flow of heat only toward the cold plate, it is unlikely that thermal leakage was entirely eliminated.

A special test was designed as a check on the accuracy of the parallel banks of thermocouples used to secure a thermal balance between the different units of the thermal conductivity apparatus. Four thermocouples were attached to the hot plate at widely separated points and the thermal electromotive forces between the various thermo-junctions noted over a period of two hours. Before beginning the test the apparatus was permitted to reach a steady state with a 5 cm. slab of bonded, ground cork between the hot and cold plates. Throughout the duration of the test the greatest temperature difference between any two thermo-junctions was 0.58° Centigrade.

Particular care was taken to maintain identical conditions in all the tests and it is thought that the results obtained are sufficiently accurate for purposes of comparison.

DATA

Thermal conductivity determinations were made on three widely different types of loose-fill thermal insulation, namely, vermiculite, rock wool, and ground cornstalks. The two different brands of heat treated mica insulation are designated as Vermiculite "A" and Vermiculite "B". Two kinds of mineral wool were tested, each at two different densities. The qualities of the two kinds of mineral wool are indicated by the terms "lumpy" and "carded" which are self-explanatory. Samples of ground cornstalks in two sizes were tested.

For the purpose of maintaining uniformity the same sample was used throughout the entire range of moisture contents used in connection with any particular type of insulation. For each sample the moisture content was increased in successive tests until the thermal conductivity reached a value comparable to that obtained for a mass of air of the same size and shape, tested under identical conditions.

All measurements discussed in this report were on samples having a thickness of 2.6 cm. and all tests were made with the sample in a vertical position. The values of the thermal conductivity, K, were calculated from the following formula which was obtained by combining equation (2), the steady state solution of the one dimensional heat flow equation, with Joule's law:

$$K = \frac{I^2 R' d}{J(T_2 - T_1)}$$

where K = thermal conductivity in cal sec^{-1} cm^{-2} $(°C.\ cm^{-1})^{-1}$.

I = current in amperes.

R' = resistance in ohms cm^{-2}. ($R' = 0.032$)

d = thickness of sample in cm.

J = electrical equivalent of heat.

$T_2 - T_1$ = temperature difference between hot and cold plates in °C.

The values of the thermal conductivities for the various types of loose-fill insulation tested are shown graphically in figures 5 to 9.

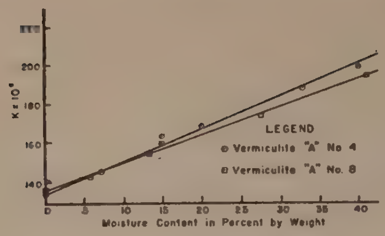

Fig. 5. Vermiculite "A"

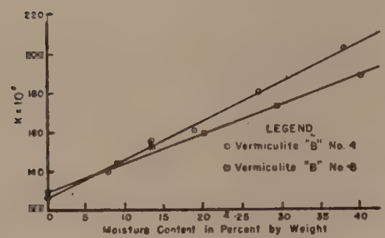

Fig. 6. Vermiculite "B"

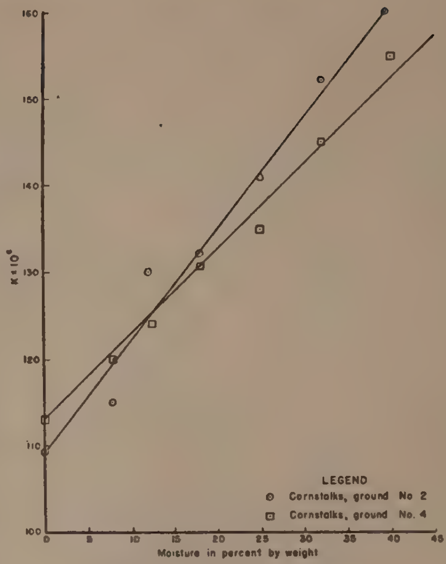

Fig. 9. Ground cornstalks

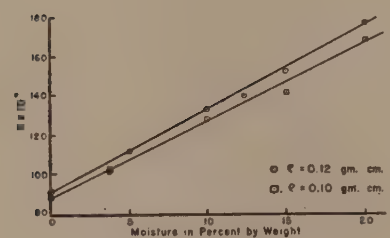

Fig. 7. Rock wool, carded

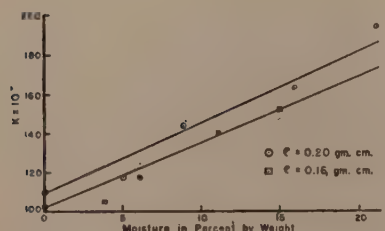

Fig. 8. Rock wool, lumpy

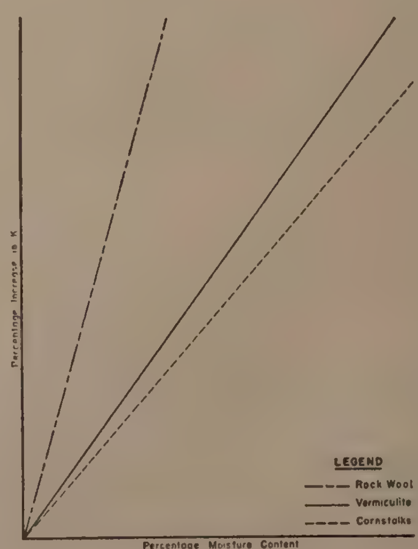

Fig. 10. Rate of change of thermal conductivity with moisture

CONCLUSIONS

Tests on the various types of loose-fill insulators reveal that there is a linear increase of thermal conductivity with increased moisture content. The rate of this increase for the three general types of insulation tested is roughly indicated in table 7 and a graphical comparison is made in figure 10.

TABLE 7.

Rate of change of thermal conductivity with moisture content.

Type of insulation	$\dfrac{\text{percentage increase in thermal conductivity}}{\text{percentage moisture content by weight}}$
Rock wool	$\dfrac{7}{2}$
Vermiculite	$\dfrac{7}{5}$
Cornstalks	$\dfrac{7}{6}$

The excessively high rate of increase for rock wool may be accounted for, at least in part, by experimental difficulties encountered while humidifying samples of this type. For the greatest efficiency, samples of rock wool insulation must consist of light, fluffy material. Agitation of the material while conditioning it to the desired degree of hygroscopic moisture content caused the mineral fibers to form into small balls or pellets. This tendency, coupled with the addition of moisture, effected quite a radical change in the mechanical structure of the rock wool samples. In practice the rock wool curve of figure 10 would doubtless have a much smaller slope.

As a basis for checking results, special tests were made with air as the insulating medium. Using the 2.6 cm. sample frame and the edge insulation employed in the regular series of tests, the thermal conductivity of air was found to be $K = 0.000177$. In another test on air the sample frame and edge insulation were removed thus giving free play to convection currents in the room. In this latter test it was impossible to secure a steady state. Readings taken over a period of three hours revealed fluctuations in the range $K = 0.000155$ to $K = 0.000189$. This value of K for air is approximately that obtained for loose-fill insulators with a moisture content of 20 to 35 per cent as shown on the curves in figures 5 to 9. Consequently, it is concluded that, for practical purposes, a hygroscopic moisture content of 20 to 35 per cent by weight completely vitiates the efficacy of loose-fill insulation.

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